# LA OTRA VISIÓN DEL ODONTOBLASTO

# UNA REVISIÓN NARRATIVA

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**PROGRAMA DE ENDODONCIA- FACULTAD DE ODONTOLOGÍA**

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## HOJA DE IDENTIFICACION

| | |
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**RESUMEN.**

**LA OTRA VISIÓN DEL ODONTOBLASTO. UNA REVISIÓN NARRATIVA**

**Antecedentes:** El odontoblasto durante muchos años ha sido descrito como una célula especializada encargada de secretar la matriz extracelular que favorece la biomineralización del complejo pulpo-dentinal, pero en la actualidad las diferentes investigaciones han demostrado que esta célula de origen ectomesenquimal cumple múltiples funciones reguladoras en los procesos fisiopatológicos del complejo dentino-pulpar. **Objetivo:** Revisar y evidenciar las múltiples funciones del odontoblasto a nivel inmunológico, vascular y neuronal como modulador de las respuestas celulares y moleculares en la regeneración y/o reparación en el complejo pulpo-dentinal. **Metodología:** Se realizó una búsqueda electrónica en bases de datos desde enero de 2009 hasta junio de 2018. Las bases de datos que se utilizaron fueron: PUBMED, EMBASE, MEDLINE, Lilacs, Sciente Direct y se incluyeron artículos en Inglés. **Resultados:** La búsqueda en la base de datos electrónica arrojo 492.157 publicaciones. Después de la evaluación de los títulos, resumen, criterios de inclusión y exclusión se seleccionaron un total de 94 artículos para la revisión. **Conclusiones:** Este estudio examinó y comparó la literatura disponible actualmente acerca de las múltiples funciones del odontoblasto como la nocicepción, la respuesta sensorial a múltiples estímulos externos, la capacidad inmunológica y la participación en procesos inflamatorios, encontrando que esta célula especializada tiene la capacidad de participar y modular los procesos fisiopatológicos tanto de la dentina como de la pulpa.

**Palabras claves:** Odontoblasto, nocicepción, inmunología, inflamación.

# ABSTRACT.

## THE OTHER PERSPECTIVE OF THE ODONTOBLAST. A NARRATIVE REVISION

**Background:** The odontoblast has been describe during years as a specialised cell in charge of secreting the extra-cellular matrix which favours the bio-mineralisation of the dentinal pulp complex. Different investigations have currently shown that this cell, of a ectomesenchyme origin has several regulating functions of physiopathological process of the dentin-pulp complex. **Objective:** to review and evidence the odontoblast's multiple functions at an immunological, vascular and neuronal level as modulator of cellular and molecular responses in the regeneration and repair of the dentin-pulp complex. **Methodology:** An electronic search was carried out of databases from January 2009 to June 2018 and the information used was: *PUBMED, EMBASE, MEDLINE, Lilacs, Science Direct* including articles in English. **Results:** the database search yielded 492.157 publications out of which 94 were selected after reviewing the titles, abstract, inclusion and exclusion criteria. **Conclusions:** This study examined and compared the currently available literature regarding multiple functions of the odontoblast such as nocioception, sensory response to external stimuli, immunological capability and inflammatory processes, finding this specialised cell has the ability to participate and modulate physio-pathological processes of the dentin and pulp.



**Key words:** odontoblast, nocioception, immunology, inflammation.

## 1. Introducción.

Cada tejido se compone de múltiples tipos de células que son evolutivas y funcionalmente integradas en la unidad que llamamos un órgano (1). El complejo dentino-pulpar, es un sistema complejo y completo en donde el órgano del diente requiere de un sistema vásculo-nervioso que favorezca la viabilidad celular que conforma dicho sistema, mediante el transporte de oxígeno, nutrientes, entre otros; y la parte sensorial, mediante las fibras nerviosas y el odontoblasto para la propicepción y modulación de las fuerzas masticatorias; por lo tanto, este complejo tiene la propiedad mecano-sensorial (2). En donde diversas células pulpares, el sistema inmune, el sistema vascular y la inervación participan conjuntamente, para conformar el complejo dentinopulpar en un un órgano funcional que puede detectarse y protegerse (3).

El tejido pulpar está rodeado y protegido de tejido biomineralizado, cuyas estructuras duras son el esmalte y la dentina, en donde si uno de estos tejidos biomineralizados se llegaran a lesionar, bien sea por un proceso infeccioso y/o trauma dento-alveolar, el complejo dentino pulpar tiene un rol importante el cual es inducir a la aposición de dentina terciaria, la cual será la dentina reaccionaria mediante el estímulo de una célula multifuncional: el odontoblasto, en donde una de sus funciones es la secreción de dentina y así proteger la pulpa dental (4, 5).

Los odontoblastos (Sasaki *et. al*., 1996), son células post mitóticas de gran longevidad, cuyo origen se da a partir de las células de la cresta neural craneal, y su ubicación está a nivel del límite entre la dentina y la pulpa, junto con el rol en la aposición de tejido biomineralizado durante toda la vida del diente. Los odontoblastos son similares a las neuronas y los cardiomiocitos, los cuales no pueden ser reemplazados (6, 7).

En la estructura de su morfología, presentan procesos citoplasmáticos los cuales se extienden hacia los túbulos dentinales formando una sola capa de cuerpos columnares y altamente polarizados, cuando están en estadío maduro presentan retículo endoplasmático liso y rugoso,

aparato de golgi y mitocondrias para poder sintetizar proteína relacionadas con la dentinogénesis, también presentan uniones gap, las cuales son principalmente las uniones connexin, podrían estar involucrado en la transducción de señales autocrinas y paracrinas (Fried *et. al*., 1996) (8, 9).

En su fase madura, los odontoblastos expresan ciertos canales iónicos como TRPV, TRPA, lo que sugiere que pueden estar relacionados con una función sensorial (Chung *et al*., 2013). Esto podría lograrse a través de comunicaciones con fibras nerviosas mediante la liberación de ATP y/o a través de interacciones con células inmunes (10, 11).

El odontoblasto durante muchos años ha sido descrito como una célula especializada encargada de secretar la matriz extracelular que favorece la biomineralización del complejo pulpo-dentinal, pero en la actualidad las diferentes investigaciones han demostrado que esta célula de origen ectomesenquimal cumple múltiples funciones reguladoras en los procesos fisiopatológicos a nivel nociceptivo, inmunológico y vascular; participando activamente en las fases de regeneración y/o reparación pulpo-dentinal (12, 13) .

Por lo tanto, este abordaje permite al profesional tener una nueva y amplia perspectiva acerca del odontoblasto y así enfocar diversas terapéuticas clínicas alternativas en el campo de la endodoncia, favoreciendo los procesos de regeneración y/o reparación tisular para el complejo pulpo-dentinal.

## 2. Antecedentes y situación actual.

Los odontoblastos son células especializadas del tejido pulpar. Ellos se derivan de las células de la cresta neural durante los estadios iniciales de desarrollo dental (Chai *et al*., 2000; Hall *et al*., 2013), se diferencian, se organizan y regulan la síntesis de tejido biomeralizado, es decir la matriz dentinaria compuesta por colágeno tipo I, proteínas no colágenas (5,6). Su diferenciación terminal tiene como característica células elongadas y altamente polarizadas con una forma de empalizada de aproximadamente 50 µm de altura (Couve, 1986), el cese de su ciclo celular, su núcleo ubicado hacia la parte proximal del cuerpo celular (2,6) y secreción de la matriz dentinal durante la formación dental y controla el proceso de biomineralización; en donde en los inicios de la biomineralización están presente vesículas matriciales que contienen iones de Calcio (Ca2+) y fosfato inorgánico, acumulándose y precipitándose en forma de cristales de hidroxiapatita. Participando proteínas SIBLINGs como son la osteopontina, sialofosfoproteína dentinal, proteína de matriz dentinaria, osteonectina y proteoglicanos como fibromodulina, osteoadherina, decorin, tienendo un rol fundamental en el proceso de la biomineralización del colágeno tipo I (14, 15).

Los cilios primarios son mecanorreceptores presentes en odontoblastos humanos, lo cual sugiere su participación en los procesos de transducción sensorial (Magloire *et al*., 2004, 2010). Se ha propuesto que funciona como una especie de antena extracelular que sensa y detecta factores tróficos; su expresión es una característica de una célula posmitótica altamente conservada. Este cilio es un axonema microtubular, tiene un único cilio, es no movil y tiene una membrana que expresa elementos de transducción de señales, tales como los complejos viales Sonic hedgehog (Shh) y Wingless (Wnt) (2).

Se ha demostrado en estudios previos, que los odontoblastos jóvenes coronales están acoplados eléctricamente mediante uniones gap, forman una organización sensorial; sugiriendo un rol en la coordinación de la función celular inter odontoblástica con respecto a la

formación y aposición de dentina; en donde las uniones gap entre odontoblastos humanos están principalmente formadas por connexin 43 (Cx43), cuando su expresión está reducida se describe como el proceso de envejecimiento de la pulpa dental humana, aparente asociado con una pérdida de vitalidad pulpar (16, 17).

Estas células tienen una función activa en el transporte de iones de Ca2+ y fosfato inorgánico al frente de biomineralización, presentando altas concentraciones de iones de $Ca^{2+}$ durante la dentinogénesis, interviniendo la célula que capta el ion $Ca^{2+}$ a través del canal de $Ca^{2+}$ tipo L dependiente de voltaje, para su posterior liberación a través de las bombas de Ca-ATPasa y Ca-Na. Con respecto al mecanismo de transporte del fosfato aún no esta dilucidado (18, 19).

Por otra parte los odontoblastos que están ubicados estratégicamente en la superficie externa del complejo pulpo-dentinal y organizados en empalizada están conectados entre sí a nivel de su polo apical por múltiples uniones tipo desmosomas, formando una barrera resistente y selectiva para poder realizar un control y así contribuir a una homeostasis entre la dentina y la pulpa en condiciones fisiológicas o patológicas. (20).

A diferencia del tejido óseo que se remodela constantemente durante la vida, la dentina no se puede remodelar y una vez que esta se pierde no puede ser reemplazada. Así mismo, el odontoblasto al percibir un estímulo nocivo (frio, calor, ácido, prepraración de una cavidad, etc) en lugares donde la prolongación odontoblastica está más cerca del área de la nocicepción, su respuesta será la secrección de la dentina terciaria reaccionaria para evitar un daño irreversible al tejido pulpar (21, 22).

Teóricamente se ha demostrado que se producen dos tipos de dentina terciaria en respuesta a agentes irritantes como la dentina reaccionaria (odontoblastos) y la dentina reparativa (células parecidas a los odontoblastos) (23).

Si la respuesta defensiva de los odontoblastos frente al estímulo nocivo no es suficiente se produce la muerte celular estas células, las cuales serán reemplazadas por el proceso de inducción de las células madre mesenquimales (MSCs) hacia el odontoblasto y se requiere de la activación de una importante vía de señalización Wnt/β-catenina, en donde este proceso hace que la β-catenina se acumule en el citoplasma y el núcleo se transloque, en donde activa genes diana aguas abajo. Endontes, la señalización de Wnt/β-catenina puede estar incrementando la cantidad de células tipo odontoblastos, mientras que los odontoblastos primarios no se ven afectados, porque son células postmitóticas (3).

Babb R *et al*., 2017, sugieren que la señalización Wnt / β-catenina funciona en sinergia con otras vías de señalización activadas en respuesta al daño tisular, por ejemplo, moléculas de señalización embebidas en los túbulos dentinales, la cuales se liberan en respuesta a trauma y/o lesión y así potencializar la producción de dentina, sugiriendo que la señalización autocrina de Wnt/β-catenina estimula la proliferación celular en respuesta al daño tisular y proporciona una explicación de la dentinogénesis reparativa (3).

Además de la dentinogénesis, la evidencia reciente sugiere e indica que los odontoblastos también tienen otras funciones como células receptoras sensoriales por ejemplo sensan la migración de patógenos al interior de los túbulos dentinales, iniciando una respuesta inmune y/o pro-inflamatoria, participando en la percepción de un estímulo nocivo por medio de canales iónicos que a su vez son capaces de sensar la estimulación mecánica (Magloire *et al*., 2010; Shibukawa *et al*., 2015; Goldberg *et al*., 2015; Nishiyama *et al*., 2016) (25).

## 3. Objetivos.

*Objetivo General.*

Evidenciar las múltiples funciones del odontoblasto a nivel inmunológico, vascular y neuronal como modulador de las respuestas celulares y moleculares en la regeneración y/o reparación en el complejo pulpo-dentinal.

*Objetivos Específicos.*

- Realizar una revisión exhaustiva en las bases de datos Scopus, Pubmed, Medline, Embase, Lilacs, Science direct para evidenciar las múltiples funciones del odontoblasto.
- Seleccionar artículos científicos de revistas indexadas y cuyos autores tenga relevancia científica en el área específica de investigación.
- Basada en la literatura evaluada, elaborar un artículo de revisión de literatura para evidenciar las múltiples funciones del Odontoblasto.

## 4. Metodología para el desarrollo de la revisión.

*a. Tipo de estudio.*

Revisión la literatura.

*b. Métodos.*

*1. Pregunta de la revisión*

**¿El odontoblasto regula los procesos fisiopatológicos relacionados con respuesta inmunológica, nocicepción e inflamación neurogénica en el complejo pulpo-dentinario?**

*2. Estructura de la revisión*

Se realizó una búsqueda electrónica de la literatura en bases de datos como PUBMED, EMBASE, Lilacs y Science Direct. Para la búsqueda se utilizaron encabezados de términos médicos (MeSH), descriptores en ciencias de la salud (DeCS) y palabras claves. Se utilizaron los operadores booleanos OR, AND.

La búsqueda comprendió artículos publicados en revistas indexadas con fechas desde enero de 2009 hasta junio de 2018. La estrategia de búsqueda completa se estableció para cada base de datos consultada, sobre la estrategia de búsqueda desarrollada para PUBMED y SCIENCE DIRECT.

*3. Búsqueda de información:*

*a. Selección de palabras claves por temática*

| Tabla 1.- SELECCIÓN DE PALABRAS CLAVES POR TEMÁTICA DE REVISIÓN | | |
|---|---|---|
| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** | |
| **Variable** | **Palabras claves** | |
| Nociception | Palabra clave | **Nociception** |
| | Términos [MeSH] ingles | Nociception |
| | Términos [DeSC]<br>español/ inglés/ portugués | Nocicepción, Nociception, Nociceptividade |
| | Sinónimos / Términos relacionados | Nociperception<br>Nociceptions<br>Nociperceptions |
| Immunology | Palabra clave | Inmunología |
| | Términos [MeSH] ingles | Immunology |
| | Términos [DeSC]<br>español/ inglés/ portugués | Inmunología<br>Immunology<br>Imunologia |
| | Sinónimos / Términos relacionados | - |

| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** | |
|---|---|---|
| **Variable** | **Palabras claves** | |
| Inflamación | Palabra clave | **Inflamación** |
| | Términos [MeSH] ingles | Inflammation |
| | Términos [DeSC] | Inflamación |

| Temática | LA OTRA VISIÓN DEL ODONTOBLASTO | |
|---|---|---|
| Variable | Palabras claves | |
| | español/ inglés/ portugués | Inflammation<br>Inflamação |
| | Sinónimos / Términos relacionados | - |

*b. Estructuración de estrategia de búsqueda por temática*

| Tabla 2. ESTRATEGIA DE BUSQUEDA | |
|---|---|
| Temática | LA OTRA VISIÓN DEL ODONTOBLASTO |
| **#1:** | Odontoblast |
| **#2:** | Odontoblast AND nociception OR sensibility |
| **#3:** | Odontoblast AND immunology OR immunology AND inflammation |
| **#4:** | #1 AND #2 |
| **#5:** | #3 AND #4 |

*c. Resultados de aplicación de estrategia de búsqueda por temática en bases de datos*

**Tabla 3.1**

**Resultados aplicación de Estrategia de búsqueda por Temática**

**PUBMED**

**Fecha: 25/06/2018 Total= 158.448**

| Temática | LA OTRA VISIÓN DEL ODONTOBLASTO | | |
|---|---|---|---|
| **Búsqueda** | **Algoritmos** | **Cantidad de artículos encontrados** | **Cantidad seleccionada por Titulo/ Abstract** |

| | | | |
|---|---|---|---|
| #1 | Odontoblast | *4.066* | 60 |
| #2 | Odontoblast AND nociception OR sensibility | 6.524 | 42 |
| #3 | Odontoblast AND immunology OR immunology AND inflammation | 141.127 | 28 |
| #4 | Odontoblast AND nociception OR sensibility | 6.524 | 42 |
| # 5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation | 207 | 15 |

**Tabla 3.2**

**Resultados aplicación de Estrategia de búsqueda por Temática**

**MEDLINE**

**Fecha: 25/06/2018 Total= 0**

| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** |
|---|---|

| **Búsqueda** | **Algoritmos** | **Cantidad de artículos encontrados** | **Cantidad seleccionada por Titulo/ Abstract** |
|---|---|---|---|
| #1 | Odontoblast | 0 | 0 |
| #2 | Odontoblast AND nociception OR sensibility | 0 | 0 |
| #3 | Odontoblast AND immunology OR immunology AND inflammation | 0 | 0 |
| #4 | Odontoblast AND nociception OR sensibility | 0 | 0 |
| # 5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation | 0 | 0 |

**Tabla 3.3**

**Resultados aplicación de Estrategia de búsqueda por Temática**

**EMBASE**

**Fecha: 08/08/2017 Total =137.004**

| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** | | |
|---|---|---|---|
| **Búsqueda** | **Algoritmos** | **Cantidad de artículos encontrados** | **Cantidad seleccionada por Titulo/ Abstract** |
| #1 | Odontoblast | 3.723 | 100 |
| #2 | Odontoblast AND nociception OR sensibility | 12.670 | 10 |
| #3 | Odontoblast AND immunology OR immunology AND inflammation | 107.758 | 0 |
| #4 | Odontoblast AND nociception OR sensibility | 12.670 | 10 |
| # 5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation | 183 | 40 |

**Tabla 3.4**

**Resultados aplicación de Estrategia de búsqueda por Temática**

**SCIENCE DIRECT**

**Fecha: 25/06/18 Total= 196.613**

| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** | | |
|---|---|---|---|
| **Búsqueda** | **Algoritmos** | **Cantidad de artículos encontrados** | **Cantidad seleccionada por Titulo/ Abstract** |
| #1 | Odontoblast | 6.522 | 40 |

| | | | |
|---|---|---|---|
| #2 | Odontoblast AND nociception OR sensibility | 48.847 | 25 |
| #3 | Odontoblast AND immunology OR immunology AND inflammation | 90.247 | 27 |
| #4 | Odontoblast AND nociception OR sensibility | 48.847 | 25 |
| # 5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation | 2.150 | 30 |

**Tabla 3.5**

**Resultados aplicación de Estrategia de búsqueda por Temática**

**LILACS / Inglés**

**Fecha: 25/06/18 Total=92**

| **Temática** | **LA OTRA VISIÓN DEL ODONTOBLASTO** | | |
|---|---|---|---|
| **Búsqueda** | **Algoritmos** | **Cantidad de artículos encontrados** | **Cantidad seleccionada por Titulo/ Abstract** |
| #1 | Odontoblast | 89 | 25 |
| #2 | Odontoblast AND nociception OR sensibility | 0 | 0 |
| #3 | Odontoblast AND immunology OR immunology AND inflammation | 2 | 2 |
| #4 | Odontoblast AND nociception OR sensibility | 0 | 0 |
| #5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation | 1 | 0 |

### d. Preselección de artículos por temática

Los artículos encontrados y preseleccionados por título o abstract se registran en la siguiente tabla.

| Tabla 4. Preselección de artículos por temática<br><br>PUBMED | |
|---|---|
| **TEMATICA** | **LA OTRA VISIÓN DEL ODONTOBLASTO** |
| **BASE DE DATOS** | **PUBMED** |
| **ALGORITMO FINAL** | Odontoblasts, Odontoblasts AND nociception, Odontoblasts AND immunology OR immunology AND inflammation. |
| **artículos preseleccionados**<br><br>Referencia -estilo Vancouver y abstract | |

**1. Couve E, Osorio R, Schmachtenberg O. The Amazing Odontoblast: Activity, Autophagy, and Aging. J Dent Res. 2013 Jun; 92 (9): 765-772.**

Odontoblasts are dentin-secreting cells that survive for the whole life of a healthy tooth. Once teeth are completely erupted, odontoblasts transform into a mature stage that allows for their functional conservation for decades, while maintaining the capacity for secondary and reactionary dentin secretion. Odontoblasts are also critically involved in the transmission of sensory stimuli from the dentin-pulp complex and in the cellular defense against pathogens. Their longevity is sustained by an elaborate autophagic-lysosomal system that ensures organelle and protein renewal. However, progressive dysfunction of this system, in part caused by lipofuscin accumulation, reduces the fitness of odontoblasts and eventually impairs their dentin maintenance capacity. Here we review the functional activities assumed by mature odontoblasts throughout life. Understanding the biological basis of age-related changes in human odontoblasts is crucial to improving tooth preservation in the elderly.

**2. Kojima Y, Kimura M, Higashikawa A, Kono K, Ando M, Tazaki M, Shibukawa Y. Potassium Currents Activated by Depolarization in Odontoblasts. Frontiers in Physiology. 2017 Dec; 1078 (8): 1-10.**

Increased intracellular free $Ca^{2+}$ concentrations elicit plasma membrane depolarization, which leads to the activation of $K^+$ currents. However, the precise properties of $K^+$ currents activated by depolarization in odontoblasts remain to be elucidated. The present study identified biophysical and pharmacological characteristics of time-dependent and voltage-activated $K^+$ currents in freshly dissociated rat odontoblasts using patch-clamp recordings in a whole-cell configuration. Using a holding potential of − 70mV, outwardly rectifying time- and voltage-dependent currents were activated by depolarizing voltage. To record pure $K^+$ conductance, we substituted $Cl^-$ in both the extracellular and intracellular solutions with gluconate$^-$ . Under these conditions, observation of $K^+$ concentration changes in the extracellular solution showed that reversal potentials of tail currents shifted according to the $K^+$ equilibrium potential. The activation kinetics of outward $K^+$ currents were relatively slow and depended on the membrane potential. Kinetics of steady-state inactivation were fitted by a Boltzmann function. The half-maximal inactivation potential was − 38mV. Tetraethylammonium chloride, 4-aminopyridine, and a -dendrotoxin inhibited outward currents in odontoblasts in a concentration-dependent manner, suggesting that rat odontoblasts express the a -subunit of the time- and voltage-dependent $K^+$ channel (Kv) subtypes Kv1.1, 1.2, and/or 1.6. We further examined the effects of Kv activity on mineralization by alizarin red and von Kossa staining. Continuous

application of tetraethylammonium chloride to human odontoblasts grown in a mineralization medium over a 21-day period exhibited a dose-dependent decrease in mineralization efficiency compared to cells without tetraethylammonium chloride. This suggests that odontoblasts functionally express voltage-dependent K+ channels that play important roles in dentin formation.

**3. Krivanek J, Adameyko I, Fried K. Heterogeneity and Developmental Connections between Cell Types Inhabiting Teeth. Frontiers in Physiology. 2017 Jun; 376 (8): 1-10.**

Every tissue is composed of multiple cell types that are developmentally, evolutionary and functionally integrated into the unit we call an organ. Teeth, our organs for biting and mastication, are complex and made of many different cell types connected or disconnected in terms of their ontogeny. In general, epithelial and mesenchymal compartments represent the major framework of tooth formation. Thus, they give rise to the two most important matrix–producing populations: ameloblasts generating enamel and odontoblasts producing dentin. However, the real picture is far from this quite simplified view. Diverse pulp cells, the immune system, the vascular system, the innervation and cells organizing the dental follicle all interact, and jointly participate in transforming lifeless matrix into a functional organ that can sense and protect itself. Here we outline the heterogeneity of cell types that inhabit the tooth, and also provide a life history of the major populations. The mouse model system has been indispensable not only for the studies of cell lineages and heterogeneity, but also for the investigation of dental stem cells and tooth patterning during development. Finally, we briefly discuss the evolutionary aspects of cell type diversity and dental tissue integration.

**4. Qin W, Gao X, Ma T, Weir M.D, Zou J, Song B, Lin Z, Schneider A, Xu H.K. Metformin Enhances the Differentiation of Dental Pulp Cells into Odontoblasts by Activating AMPK Signaling. 2018 Abr; 44 (4): 576-584.**

Introduction: Metformin is a first-line drug for treating type 2 diabetes that regulates the differentiation of mesenchymal stem cells. Its effects on human dental pulp cells (DPCs) remain unknown. This study aimed to investigate the effects of metformin on the proliferation and differentiation of DPCs. Methods: A live/dead viability assay kit was used to examine the effects of metformin on the cell viability of DPCs. Cell proliferation was analyzed using a cell counting kit (CCK-8; Dojindo, Tokyo, Japan). Levels of phosphorylated and unphosphorylated adenosine 50-monophosphate-activated protein kinase (AMPK) were quantified by Western blot analysis in response to metformin and the AMPK signaling inhibitor Compound C (EMD Chemicals, San Diego, CA). The effects of Compound C on the metformin-induced odontoblast differentiation of DPCs were determined by alkaline phosphatase activity assay and von Kossa staining, and the expression of odontoblastic markers was evaluated by reverse-transcription polymerase chain reaction analysis. Results: DPCs exhibited mesenchymal stem cell characteristics using flow cytometry. Different doses ofmetformin were shown to be cytocompatible with DPCs, yielding >90% cell viability. None of the concentrations of metformin up to 50 mmol/L affected cell proliferation. The Western blot assay showed that DPCs express functional organic catión transporter 1, a transmembrane protein that mediates the intracellular uptake of metformin. Metformin significantly activated the AMPK pathway in a dose-dependent manner. In addition, it stimulated alkaline phosphatase activity; enhanced mineralized nodule formation; and increased the expression of odontoblastic markers including dentin sialophosphoprotein, dentin matrix protein 1, runt-related transcription factor 2, and osteocalcin. Moreover, pretreatment with Compound C, a specific AMPK inhibitor, markedly reversed metformin-

induced odontoblastic differentiation and cell mineralization. Conclusions: This study shows that metformin can induce DPC differentiation and mineralization in an AMPK dependent manner and that this well-tolerated antidiabetic drug has potential in regenerative endodontics as well as in other regenerative applications.

5. **Goldberg M, Njeh A, Uzunoglu E. Is Pulp Inflammation a Prerequisite for Pulp Healing and Regeneration?. Mediators Inflamm. 2015 Oct; 347649.**

The importance of inflammation has been underestimated in pulpal healing, and in the past, it has been considered only as an undesirable effect. Associated with moderate inflammation, necrosis includes pyroptosis, apoptosis, and nemosis. There are now evidences that inflammation is a prerequisite for pulp healing, with series of events ahead of regeneration. Immunocompetent cells are recruited in the apical part. They slide along the root and migrate toward the crown. Due to the high alkalinity of the capping agent, pulp cells display mild inflammation, proliferate, and increase in number and size and initiate mineralization. Pulp fibroblasts become odontoblast-like cells producing type I collagen, alkaline phosphatase, and SPARC/osteonectin. Molecules of the SIBLING family, matrix metalloproteinases, and vascular and nerve mediators are also implicated in the formation of a reparative dentinal bridge, osteo/orthodentin closing the pulpexposure. Beneath a calciotraumatic line, a thin layer identified as reactionary dentin underlines the periphery of the pulp chamber. Inflammatory and/or noninflammatory processes contribute to produce a reparative dentinal bridge closing the pulp exposure, with minute canaliculi and large tunnel defects. Depending on the form and severity of the inflammatory and noninflammatory processes, and according to the capping agent, pulp reactions are induced specifically.

6. **Kawashima N, Okiji T. Odontoblasts: Specialized hard-tissue-forming cells in the dentin-pulp complex. Congenital Anomalies. 2016 Abr; 56, 144–153.**

Odontoblasts are specialized cells that produce dentin and exhibit unique morphological characteristics; i.e., they extend cytoplasmic processes into dentinal tubules. While osteoblasts, which are typical hard-tissue-forming cells, are generated from mesenchymal stem cells during normal and pathological bone metabolism, the induction of odontoblasts only occurs once during tooth development, and odontoblasts survive throughout the lives of healthy teeth. During the differentiation of odontoblasts, signaling molecules from the inner enamel epithelium are considered necessary for the differentiation of odontoblast precursors, i.e., peripheral dental papilla cells. If odontoblasts are destroyed by severe external stimuli, such as deep caries, the differentiation of dental pulp stem cells into odontoblast-like cells is induced. Various bioactive molecules, such as non-collagenous proteins, might be involved in this process, although the precise mechanisms responsible for odontoblast differentiation have not been fully elucidated. Recently, our knowledge about the other functional activities of odontoblasts (apart from dentin formation) has increased. For example, it has been suggested that odontoblasts might act as nociceptive receptors, and surveillance cells that detect the invasion of exogenous pathogens. The regeneration of the dentin-pulp complex has recently gained much attention as a promising future treatment modality that could increase the longevity of pulpless teeth. Finally, congenital dentin anomalies, which are concerned with the disturbance of odontoblast functions, are summarized.

7. **Babb R, Chandrasekaran D, Carvalho V, Sharpe P. Axin2-expressing cells differentiate into reparative odontoblasts via autocrine Wnt/β-catenin signaling in response to tooth damage. Sci Rep. 2017 Jun 8;7(1):3102.**

In non-growing teeth, such as mouse and human molars, primary odontoblasts are long-lived postmitotic cells that secrete dentine throughout the life of the tooth. New odontoblast-like cells are only produced in response to a damage or trauma. Little is known about the molecular events that initiate mesenchymal stem cells to proliferate and differentiate into odontoblast-like cells in response to dentine damage. The reparative and regenerative capacity of multiple mammalian tissues depends on the activation of Wnt/β-catenin signaling pathway. In this study, we investigated the molecular role of Wnt/β-catenin signaling pathway in reparative dentinogenesis using an in vivo mouse tooth damage model. We found that Axin2 is rapidly upregulated in response to tooth damage and that these Axin2-expressing cells differentiate into new odontoblast-like cells that secrete reparative dentine. In addition, the Axin2-expressing cells produce a source of Wnt that acts in an autocrine manner to modulate reparative dentinogenesis.

**8. Song Y, Cao P, Gu Z, Xiao J, Lian M, Huang D, Xing J, Zhang Y, Feng X, Wang C. The Role of Neuropilin-1–FYN Interaction in Odontoblast Differentiation of Dental Pulp Stem Cells. Cell Reprogram. 2018 Apr; 20(2):117-126.**

Abnormal odontoblast differentiation of dental pulp stem cells (DPSCs) caused by inflammation is closely related to the development of dental caries. Neuropilin-1 (NRP1) is one of the members of neuropilin family. It can combine with disparate ligands involved in regulating cell differentiation. FYN belongs to the proteintyrosine kinase family, which has been implicated in the control of cell growth, and the effect can be further strengthened by inflammatory factors. In our studies, we verified that NRP1 can form complexes with FYN and have the correlation changes in odontoblast differentiation of DPSCs. Therefore, we surmise that in the progress of dental caries, NRP1 interacts with FYN, by expanding inflammation and inhibition of odontoblast differentiation of DPSCs through nuclear factor kappa B (NF-jB) signaling pathway. In this subject, we first investigated the expression and interaction of NRP1 and FYN in DPSCs. And then, we researched the effect of this complex controlling downstream signal pathway in normal or inflammation stimulated DPSCs. Finally, we analyzed the relationship between this role and odontoblast differentiation of DPSCs. This research will provide the molecular mechanism of inflammation factors of dental caries through activating NF-jB signal regulating odontoblast differentiation in DPSCs for finding new potential drug targets for the clinical treatment of dental caries.

**9. Solé-Magdalena A, Revuelta EG, Menénez-Díaz I, Calavia MG, Cobo T, García-Suárez O, Pérez-Piñera P, De Carlos F, Cobo J, Vega JA. Human odontoblasts express transient receptor protein and acid-sensing ion channel mechanosensor proteins. Microsc Res Tech. 2011 May;74(5):457-63.**

Diverse proteins of the denegerin/epithelial sodium channel (DEG/ENa(+) C) superfamily, in particular those belonging to the acid-sensing ion channel (ASIC) family, as well as some members of the transient receptor protein (TRP) channel, function as mechanosensors or may be required for mechanosensation in a diverse range of species and cell types. Therefore, we investigated the putative mechanosensitive function of human odontoblasts using immunohistochemistry to detect ENa(+) C subunits (α, β, and γ) and ASIC (1, 2, 3, and 4) proteins, as well as TRPV4, in these cells. Positive and specific immunoreactivity in the odontoblast soma and/or processes was detected for all proteins studied except α-ENa(+) C. The intensity of immunostaining was high for β-ENa(+) C and ASIC2, whereas it was low for ASIC1, ASIC3, γ-ENa(+) C, and TRPV4, being absent for α-ENa(+) C and ASIC4. These results suggest that human odontoblasts in

situ express proteins related to mechanosensitive channels that probably participate in the mechanisms involved in teeth sensory transmission.

**10. Magloire H, Couble ML, Thivichon-Prince B, Maurin JC, Bleicher F. Odontoblast: a mechano sensory cell. J Exp Zool B Mol Dev Evol. 2009 Jul 15;312B(5):416-24.**

Odontoblasts are organized as a single layer of specialized cells responsible for dentine formation and presumably for playing a role in tooth pain transmission. Each cell has an extension running into a dentinal tubule and bathing in the dentinal fluid. A dense network of sensory unmyelinated nerve fibers surrounds the cell bodies and processes. Thus, dentinal tubules subjected to external stimuli causing dentinal fluid movements and odontoblasts/nerve complex response may represent a unique mechano-sensory system giving to dentine-forming cells a pivotal role in signal transduction. Mediators of mechano-transduction identified in odontoblast include mechano-sensitive ion channels (high conductance calcium-activated potassium channel--K(Ca)--and a 2P domain potassium channel--TREK-1) and primary cilium. In many tissues, the latter is essential for microenvironment sensing but its role in the control of odontoblast behavior remains to be elucidated. Recent evidence for excitable properties and the concentration of key channels to the terminal web suggest that odontoblasts may operate as sensor cells.

**11. Kimura M, Nishi K, Higashikawa A, Ohyama S, Sakurai K, Tazaki M, Shibukawa Y. High pH-Sensitive Store-Operated $Ca^{2+}$ Entry Mediated by $Ca^{2+}$ Release-Activated $Ca^{2+}$ Channelsin Rat Odontoblasts. Front Physiol. 2018 May 1;9:443.**

Odontoblasts play a crucial role in dentin formation and sensory transduction following the application of stimuli to the dentin surface. Various exogenous and endogenous stimuli elicit an increase in the intracellular free calcium concentration ($[Ca^{2+}]_i$) in odontoblasts, which is mediated by $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores and/or $Ca^{2+}$ influx from the extracellular medium. In a previous study, we demonstrated that the depletion of $Ca^{2+}$ stores in odontoblasts activated store-operated $Ca^{2+}$ entry (SOCE), a $Ca^{2+}$ influx pathway. However, the precise biophysical and pharmacological properties of SOCE in odontoblasts have remained unclear. In the present study, we examined the functional expression and pharmacological properties of $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels that mediate SOCE and evaluated the alkali sensitivity of SOCE in rat odontoblasts. In the absence of extracellular $Ca^{2+}$, treatment with thapsigargin (TG), a sarco/endoplasmic reticulum $Ca^{2+}$-ATPase inhibitor, induced an increase in $[Ca^{2+}]_i$. After $[Ca^{2+}]_i$ returned to near-resting levels, the subsequent application of 2.5 mM extracellular $Ca^{2+}$ resulted in an increase in $[Ca^{2+}]_i$ which is a typical of SOCE activation. Additionally, application of 2-methylthioadenosine diphosphate trisodium salt (2-MeSADP), a $P2Y_{1,12,13}$ receptor agonist, or carbachol (CCh), a muscarinic cholinergic receptor agonist, in the absence of extracellular $Ca^{2+}$, induced a transient increase in $[Ca^{2+}]_i$. The subsequent addition of extracellular $Ca^{2+}$ resulted in significantly higher $[Ca^{2+}]_i$ in 2-MeSADP- or CCh-treated odontoblasts than in untreated cells. SOCE, that is activated by addition of extracellular $Ca^{2+}$ in the TG pretreated odontoblasts was then suppressed by Synta66, BTP2, or lanthanum, which are CRAC channel inhibitors. Treatment with an alkaline solution enhanced SOCE, while treatment with HC030031, a TRPA1 channel antagonist, inhibited it. The amplitude of SOCE at pH 9 in the presence of HC030031 was higher than that at pH 7.4 in the absence of HC030031. These findings indicate that CRAC channel-mediated alkali-sensitive SOCE occurs in odontoblasts. SOCE is mediated by P2Y and muscarinic-cholinergic receptors, which are activated by endogenous ligands in odontoblasts.

**12. Farges JC, Alliot-Licht B, Baudouin C, Msika P, Bleicher F, Carrouel F. Odontoblast control of dental pulp inflammation triggered by cariogenic bacteria. Front Physiol. 2013 Nov 11;4:326.**

Inflammation is part of the normal protective immune response of the host to tissue infection. It promotes the recruitment

of circulating immunocompetent blood cells and their migration through the endothelial barrier to gain access to the damaged site and eliminate injurious pathogens. If kept uncontrolled, inflammation can result in a wide range of acute, chronic, and systemic inflammatory disorders. Therefore, higher organisms have evolved protective mechanisms to ensure the inflammatory response is resolved in a specific time-limited manner. Resolution of inflammation requires the elimination of injurious agents and the removal of pro-inflammatory mediators that initiate host defense against microbial invasion. In addition, anti-inflammatory agents including steroids, IL-1 receptor antagonist, soluble TNF receptor, interleukin-10 (IL-10), nitric oxide (NO), heme oxygenase-1, as well as regulatory T lymphocytes (Tregs), are produced to limit tissue damage and promote return to homeostasis a major cause of inflammation in human dental pulp is the presence, in the affected dentine, of the oral bacteria responsible for carious lesion development.

13. **Farges JC, Bellanger A, Ducret M, Aubert-Foucher E, Richard B, Alliot-Licht B, Bleicher F, Carrouel F. Human odontoblast-like cells produce nitric oxide with antibacterial activity upon TLR2 activation. Front Physiol. 2015 Jun 23;6:185.**

The penetration of cariogenic oral bacteria into enamel and dentin during the caries process triggers an immune/inflammatory response in the underlying pulp tissue, the reduction of which is considered a prerequisite to dentinogenesis-based pulp regeneration. If the role of odontoblasts in dentin formation is well known, their involvement in the antibacterial response of the dental pulp to cariogenic microorganisms has yet to be elucidated. Our aim here was to determine if odontoblasts produce nitric oxide (NO) with antibacterial activity upon activation of Toll-like receptor-2 (TLR2), a cell membrane receptor involved in the recognition of cariogenic Gram-positive bacteria. Human odontoblast-like cells differentiated from dental pulp explants were stimulated with the TLR2 synthetic agonist Pam2CSK4. We found that NOS1, NOS2, and NOS3 gene expression was increased in Pam2CSK4-stimulated odontoblast-like cells compared to unstimulated ones. NOS2 was the most up-regulated gene. NOS1 and NOS3 proteins were not detected in Pam2CSK4-stimulated or control cultures. NOS2 protein synthesis, NOS activity and NO extracellular release were all augmented in stimulated samples. Pam2CSK4-stimulated cell supernatants reduced Streptococcus mutans growth, an effect counteracted by the NOS inhibitor L-NAME. In vivo, the NOS2 gene was up-regulated in the inflamed pulp of carious teeth compared with healthy ones. NOS2 protein was immunolocalized in odontoblasts situated beneath the caries lesion but not in pulp cells from healthy teeth. These results suggest that odontoblasts may participate to the antimicrobial pulp response to dentin-invading Gram-positive bacteria through NOS2-mediated NO production. They might in this manner pave the way for accurate dental pulp healing and regeneration.

14. **Korkmaz Y, Lang H, Beikler T, Cho B, Behrends S, Bloch W, Addicks K, Raab WH. Irreversible inflammation is associated with decreased levels of the alpha1-, beta1-, and alpha2-subunits of sGC in human odontoblasts. J Dent Res. 2011 Apr;90(4):517-22.**

The nitric oxide (NO) receptor enzyme soluble guanylate cyclase (sGC) contains one prosthetic heme group as an αβ heterodimer, and two heterodimer isoforms (α(1)β(1), α(2)β(1)) were characterized to have enzyme activity. To test the irreversible inflammation-dependent regulation of sGC in odontoblasts, we incubated decalcified frozen sections of healthy and inflamed human third molars with antibodies against β-actin, nitrotyrosine, inducible nitric oxide synthase (iNOS), α(1)-, β(1)-, and α(2)-subunits of sGC and analyzed them at protein levels by quantitative immunohistochemistry. The irreversible inflammation induced an increase in the signal intensities for nitrotyrosine and iNOS and a decrease for the α(1)-, β(1)-, and α(2)-subunits of sGC in odontoblasts. Inflammatory mediators, reactive oxygen, and nitrogen species may impair the expression of the α(1)-, β(1)-, and α(2)-subunits in odontoblasts. The decrease of sGC at the protein level in inflamed odontoblasts is compatible with a critical role for sGC to mediate biological effects of NO in health.

**artículos relacionados encontrados**

Listado de artículos Referencia -estilo Vancouver y abstract

1. **Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, Cooper PR. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015 Oct; 230251.**

Dental caries is a chronic infectious disease resulting from the penetration of oral bacteria into the enamel and dentin. Microorganisms subsequently trigger inflammatory responses in the dental pulp. These events can lead to pulp healing if the infection is not too severe following the removal of diseased enamel and dentin tissues and clinical restoration of the tooth. However, chronic inflammation often persists in the pulp despite treatment, inducing permanent loss of normal tissue and reducing innate repair capacities. For complete tooth healing the formation of a reactionary/reparative dentin barrier to distance and protect the pulp from infectious agents and restorative materials is required. Clinical and in vitro experimental data clearly indicate that dentin barrier formation only occurs when pulp inflammation and infection are minimised, thus enabling reestablishment of tissue homeostasis and health. Therefore, promoting the resolution of pulpinflammation may provide a valuable therapeutic opportunity to ensure the sustainability of dental treatments. This paper focusses on key cellular and molecular mechanisms involved in pulp responses to bacteria and in the pulpal transition between caries-induced inflammation and dentinogenic-based repair. We report, using selected examples, different strategies potentially used by odontoblasts and specialized immune cells to combat dentin-invading bacteria in vivo.

**2. Jiang W, Lv H, Wang H, Wang D, Sun S, Jia Q, Wang P, Song B, Ni L. Activation of the NLRP3/caspase 1 inflammasome in human dental pulp tissue and human dental pulp fibroblasts. Cell Tissue Res. 2015 Aug; 361(2):541-55.**

The NLRP3/caspase-1 inflammasome pathway plays an important role in cellular immune defence against bacterial infection; however, its function in human dental pulp tissue and human dental pulp fibroblasts remains poorly understood. We demonstrate that NLRP3 protein expression occurs to a greater extent in pulp tissue with irreversible pulpitis than in normal pulp tissue and in tissue with reversible pulpitis. Caspase-1 is present in its active (cleaved) form only in pulp tissue with irreversible pulpitis. NLRP3 and caspase-1 are expressed in the odontoblast layers in normal human dental pulp tissue, whereas in inflamed pulp tissue, the odontoblast layers are disrupted and dental pulpcells are positive for NLRP3 and caspase-1. Additionally, we investigate the role of the NLRP3/caspase-1 inflammasome pathway in humandental pulp fibroblasts and show that ATP activates the P2X7 receptor on the cell membrane triggering K(+) efflux and inducing the gradual recruitment of the membrane pore pannexin-1. Extracellular lipopolysaccharide is able to penetrate the cytosol and activate NLRP3. Furthermore, the low intracellular K(+) concentration in the cytosol triggers reactive oxygen species generation, which also induces the NLRP3 inflammasome. Thus, the NLRP3/caspase-1 pathway has a biological role in the innate immune response mounted by human dentalpulp fibroblasts.

**3. He W, Zhang Y, Zhang J, Yu Q, Wang P, Wang Z, Smith AJ. Cytidine-phosphate-guanosine oligonucleotides induce interleukin-8 production through activation of TLR9, MyD88, NF-κB, and ERK pathways in odontoblast cells. J Endod. 2012 Jun; 38(6):780-5.**

Introduction: Odontoblasts are involved in innate immunity against invading microorganisms. However, the mechanisms of host inflammatory responses to bacterial DNA in odontoblasts are not fully understood. The purpose of this study was to investigate whether microbial cytidine-phosphate-guanosine (CpG) DNA influences interleukin-8 (IL-8) expression in odontoblasts and the signaling pathways involved. Methods: The effect of CpG oligonucleotide (CpG ODN) on IL-8 mRNA and protein expression levels in the mouse odontoblast-like cell line MDPC-23 was investigated by real-time polymerase chain reaction (PCR) analysis and enzyme-linked immunosorbent assay (ELISA). Whether Toll-like receptor

9 (TLR9), myeloid differentiation marker 88 (MyD88), nuclear factor kappa B (NF-κB), or mitogen-activated protein kinase (MAPK) pathways were involved in the CpG ODN-induced IL-8 expression was determined by examined real-time PCR, ELISA, and luciferase activity assay. Extracellular signal-regulated kinase (ERK) activation and TLR9 protein expression were measured by Western blot analysis. Results: Exposure to CpG ODN induced significant up-regulation of IL-8 mRNA and protein in MDPC-23 cells. CpG ODN-induced IL-8 up-regulation was attenuated by TLR9 inhibitor (chloroquine) and MyD88 inhibitory peptide. CpG ODN also increased the expression of TLR9mRNA and protein in MDPC-23 cells. Treatment of MDPC-23 cells with NF-κB inhibitors (pyrrolidine dithiocarbamate), IκBα phosphorylation inhibitors (Bay 117082), or IκB protease inhibitor (L-1-tosylamido-2-phenylethyl chloromethyl ketone) decreased CpG ODN-induced IL-8 expression. Furthermore, stimulation of cells with CpG ODN enhanced κB-luciferase activity, and the activity was diminished by the overexpression of dominant negative mutants of MyD88 and IκBα. In addition, CpG ODN-induced IL-8 expression was markedly suppressed by U0126, but not by SB203580 and SP600125. Moreover, CpG ODN activated ERK phosphorylation in a time-dependent manner. Conclusions: These data demonstrate that CpG ODN-induced IL-8 expression was mediated through TLR9, MyD88, NF-κB, and ERK pathways in MDPC-23 cells and suggest a possible role for the CpG DNA-mediated immune response in odontoblasts with involvement of TLR9, MyD88, and ERK pathways in this process.

**4. Hosokawa Y, Hirao K, Yumoto H, Washio A, Nakanishi T, Takegawa D, Kitamura C, Matsuo T. Functional Roles of NOD1 in Odontoblasts on Dental Pulp Innate Immunity. Biomed Res Int. 2016 Sep; 9325436.**

Caries-related pathogens are first recognized by odontoblasts and induce inflammatory events that develop to pulpitis. Generally, initial sensing of microbial pathogens is mediated by pattern recognition receptors, such as Toll-like receptor and nucleotide-binding oligomerization domain (NOD); however, little is known about NODs in odontoblasts. In this study, the levels of NODs expressed in rat odontoblastic cell line, KN-3, were assessed by flow cytometry and the levels of chemokines in NOD-specific ligand-stimulated KN-3 cells were analyzed by real-time PCR and ELISA. The signal transduction pathway activated with NOD-specific ligand was assessed by blocking assay with specific inhibitors and reporter assay. In KN-3 cells, the expression level of NOD1 was stronger than that of NOD2 and the production of chemokines, such as CINC-1, CINC-2, CCL20, and MCP-1, was upregulated by stimulation with NOD1-specific ligand, but not with NOD2-specific ligand. CINC-2 and CCL20 production by stimulation with NOD1-specific ligand was reduced by p38 MAPK and AP-1 signaling inhibitors. Furthermore, the reporter assay demonstrated AP-1 activation in NOD1-specific ligand-stimulated KN-3 cells. These findings indicated that NOD1 expressed in odontoblasts functions to upregulate the chemokines expression via p38-AP-1 signaling pathway and suggested that NOD1 may play important roles in the initiation and progression of pulpitis.

**5. Bleicher F. Odontoblast Physiology. Experimental Cell Reserch. 2014 Jul; 325 (2): 65-71.**

Odontoblasts are post-mitotic cells organized as a layer of palisade cells along the interface between the dental pulp and dentin. They are responsible for the formation of the physiological primary and secondary dentins. They synthesize the organic matrix of type I collagen and actively participate to its mineralization by secreting proteoglycans and non-collagenous proteins that are implicated in the nucleation and the control of the growth of the mineral phase. They also participate to the maintenance of this hard tissue throughout the life of the tooth by synthesizing reactionary dentin in response to pathological conditions (caries, attrition, erosion…). Besides these fundamental dentinogenic activities,

odontoblasts were recently suspected to play a role as sensor cells. They are able to sense the bacteria invasion during caries and then to initiate the pulp immune and inflammatory response. They are also well equipped in ion channels implicated in mechano transduction or nociception which make odontoblasts suitable candidates to sense external stimuli and to mediate tooth pain sensation.

**6. Farges JC , Keller JF , Carrouel F , Durand SH , Romeas A, Bleicher F, Lebecque S , Staquet MJ . Odontoblasts in the dental pulp immune response. J Exp Zool B Mol Dev Evol. 2009 Jul; 312B(5):425-36.**

Recent studies have demonstrated that human dental pulp cells sense pathogens and elicit innate and/or adaptive immunity. Particular attention has been paid to odontoblasts that are situated at the pulp-dentin interface and constitute the first line of defense to cariogenic bacteria entering dentin after enamel disruption. In this review, recent in vitro and in vivo data suggesting that odontoblasts initiate immune/inflammatory events within the dental pulp in response to cariogenic bacteria are discussed. These data include sensing of pathogens by Toll-like receptors (TLRs), production of chemokines upon cell stimulation with microbial by-products and induction of dendritic cell migration. Additional results presented here reveal that all TLR genes are expressed in the healthy human dental pulp that is thus well equipped to combat pathogens entering the tissue. Seventeen chemokine genes including CXCL12, CCL2, CXCL9, CX3CL1, CCL8, CXCL10, CCL16, CCL5, CXCL2, CCL4, CXCL11 and CCL3, and 9 chemokine receptor genes including CXCR4, CCR1, CCR5, CX3CR1, CCR10 and CXCR3, are also expressed in pulp. TLR2, CCL2 and CXCL1 are upregulated in odontoblasts both under caries lesions and upon stimulation with pathogen by-products. These molecules thus appear as preferential targets for the design of therapeutic agents able to reduce the immune/inflammatory response to cariogenic bacteria and favor pulp healing.

**7. Jang JH, Shin HW, Lee JM, Lee HW, Kim EC, Park SH. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. Mediators Inflamm. 2015 Oct;794143.**

Pathogen recognition receptors (PRRs) are a class of germ line-encoded receptors that recognize pathogen-associated molecular patterns (PAMPs). The activation of PRRs is crucial for the initiation of innate immunity, which plays a key role in first-line defense until more specific adaptive immunity is developed. PRRs differ in the signaling cascades and host responses activated by their engagement and in their tissue distribution. Currently identified PRR families are the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), the nucleotide-binding oligomerization domain-like receptors (NLRs), the retinoic acid-inducible gene-I-like receptors (RLRs), and the AIM2-like receptor (ALR). The environment of the dental pulp is substantially different from that of other tissues of the body. Dental pulp resides in a low compliance root canal system that limits the expansion of pulpal tissues during inflammatory processes. An understanding of the PRRs in dental pulp is important for immunomodulation and hence for developing therapeutic targets in the field of endodontics. Here we comprehensively review recent finding on the PRRs and the mechanisms by which innate immunity is activated. We focus on the PRRs expressed on dental pulp and periapical tissues and their role in dental pulp inflammation.

**8. Staquet MJ[1], Carrouel F, Keller JF, Baudouin C, Msika P, Bleicher F, Kufer TA, Farges JC. Pattern-recognition receptors in pulp defense. Adv Dent Res. 2011 Jul; 23(3):296-301.**

Initial sensing of infection is mediated by germline-encoded pattern-recognition receptors (PRRs), the activation of which leads to the expression of inflammatory mediators responsible for the elimination of pathogens and infected cells. PRRs act as immune sensors that provide immediate cell responses to pathogen invasion or tissue injury. Here, we review the expression of PRRs in human dental pulp cells, namely, receptors from the Toll-like (TLR) and Nod-like NLR families, by which cells recognize bacteria. Particular attention is given to odontoblasts, which are the first cells encountered by pathogens and represent, in the tooth, the first line of defense for the host. Understanding cellular and molecular mechanisms associated with the recognition of bacterial pathogens by odontoblasts is critical for the development of therapeutic strategies that aim at preventing excessive pulp inflammation and related deleterious effects.

**9. Horst OV, Tompkins KA, Coats SR, Braham PH, Darveau RP, Dale BA. TGF-β1 Inhibits TLR-mediated Odontoblast Responses to Oral Bacteria. J Dent Res. 2009 Apr; 88(4):333-8.**

TGF- β 1 exerts diverse functions in tooth development and tissue repair, but its role in microbial defenses of the tooth is not well-understood. Odontoblasts extending their cellular processes into the dentin are the first cells to recognize signals from TGF-beta1 and bacteria in carious dentin. This study aimed to determine the role of TGF-beta1 in modulating odontoblast responses to oral bacteria. We show that these responses depend upon the expression levels of microbial recognition receptors TLR2 and TLR4 on the cell surface. Porphyromonas gingivalis, Prevotella intermedia, and Fusobacterium nucleatum activated both TLRs, but TLR4 played a greater role. Lack of cell-surface TLR2 was associated with poor response to Streptococcus mutans, Enterococcus faecalis, and Lactobacillus casei. TGF-beta1inhibited TLR2 and TLR4 expression and attenuated odontoblast responses. Our findings suggest that the balance between TLR-mediatedinflammation and TGF-beta1 anti-inflammatory activity plays an important role in pulpal inflammation.

**10. Keller JF, Carrouel F, Staquet MJ, Kufer TA, Baudouin C, Msika P, Bleicher F, Farges JC. Expression of NOD2 is increased in inflamed human dental pulps and lipoteichoic acid-stimulated odontoblast-like cells. Innate Immun. 2011 Feb; 17(1):29-34.**

Human odontoblasts trigger immune response s to oral bacteria that invade dental tissues during the caries process. To date, their ability to regulate the expression of the nucleotide-binding domain leucine-rich repeat containing receptor NOD2 when challenged by Gram-positive bacteria is unknown. In this study, we investigated NOD2 expression in healthy and inflamed human dental pulps challenged by bacteria, and in cultured odontoblast-like cells stimulated with lipoteichoic acid (LTA), a Toll-like receptor (TLR) 2 agonist which is specific for Gram-positive bacteria. We found that NOD2 gene expression was significantly up-regulated in pulps with acute inflammation compared to healthy ones. In vitro, LTA augmented NOD2 gene expression and protein level in odontoblast-like cells. The increase was more pronounced in odontoblast-like cells compared to dental pulp fibroblasts. Blocking experiments in odontoblast-like cells with anti-TLR2 antibody strongly reduced the NOD2 gene expression increase, whereas stimulation with the synthetic TLR2 ligand Pam(2)CSK(4) confirmed NOD2 gene up-regulation following TLR2 engagement. These data suggest that NOD2 up-regulation is part of the odontoblast immune response to Gram-positive bacteria and might be important in protecting human dental pulp from the deleterious effects of cariogenic pathogens.

**11. He W, Qu T, Yu Q, Wang Z, Wang H, Zhang J, Smith AJ. Lipopolysaccharide enhances decorin expression through the Toll-**

**like receptor 4, myeloiddifferentiating factor 88, nuclear factor-kappa B, and mitogen activated protein kinase path ways in odontoblast cells. J Endod. 2012 Apr; 38(4):464-9.**

Introduction: Lipopolysaccharide (LPS) has been shown to regulate the function of odontoblasts. However, the molecular mechanisms of the effect of LPS on odontoblasts are poorly understood. Decorin (DCN), one of the major matrix proteoglycans, is known to affect the mineralization of teeth. In this study, we investigated whether LPS can regulate the expression of DCN in odontoblasts and determined the intracellular signaling pathways triggered by LPS. Methods: The DCN messenger RNA and protein expression changes in mouse odontoblast-lineage cells (OLCs) were detected by real-time polymerase chain reaction (PCR) analysis and enzyme-linked immunosorbent assay (ELISA). Whether TLR4, myeloid differentiatingfactor 88 (MyD88), nuclear factor-kappa B (NF-κB), or mitogen-activated protein kinase (MAPK) pathways were involved in the LPS-induced DCN expression was determined by examined real-time PCR, ELISA, and luciferase activity assay. The activation of extracellular signal-regulated kinase (ERK), p38, and JNK in OLCs was measured by Western blot analysis. Results: We found that the mouse OLCs expressed DCN. DCN messenger RNA was rapidly induced by LPS in a time- and dose-dependent manner. Pretreatment with a MyD88 inhibitory peptide, a TLR4 antibody, or a specific inhibitor for NF-κB or I Kappa B alpha (IκBα) significantly inhibited LPS-induced DCN expression. Moreover, the LPS-mediated increase in κB-luciferase activity in OLCs was suppressed by the overexpression of dominant negative mutants of TLR4, MyD88, and IκBα but not by a dominant negative mutant of TLR2. In addition, LPS stimulation activated the ERK, p38, and JNK MAPK pathways. The pretreatment of OLCs with specific inhibitors of the ERK, p38, and JNK MAPK pathways markedly offset the LPS-induced up-regulation of DCN expression. Conclusions: Our results show that LPS stimulation can up-regulate the gene expression of DCN via the TLR4, MyD88, NF-κB, and MAPK pathways in odontoblast cells.

**12. Liu Y, Gao Y, Zhan X, Cui L, Xu S, Ma D, Yue J, Wu B, Gao J. TLR4 activation by lipopolysaccharide and Streptococcus mutans induces differential regulationof proliferation and migration in human dental pulp stem cells. J Endod. 2014 Sep; 40(9):1375-81.**

Introduction: Dental pulp stem cells (DPSCs) are suspected to be an important part of the innate immune response of dental pulp, which is triggered by microorganisms that progressively invade the human tooth during the formation of caries. This study was performed to elucidate the expression of toll-like receptor 4 (TLR4) in dental pulp of deep caries and to determine whether TLR4 modulates the proliferation and migration of DPSCs. Methods: Pulp tissue samples were collected from freshly extracted human wisdom tooth. Immunohistochemistry and immunofluorescence were performed to determine the distribution of TLR4 in healthy dental pulp and dental pulp in deep caries. DPSCs were cultured and purified by collecting multiple colonies. The proliferation and migration of DPSCs were examined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide, scratch test, and transwell migration assay after stimulation with lipopolysaccharide and extracts from Streptococcusmutans. TLR4 messenger RNA (mRNA) and cytokine mRNA were evaluated with real-time polymerase chain reaction; TLR4 protein was examined with Western blot and immunocytochemistry. Results: TLR4 is expressed in the odontoblast layer and areas that colocalize with blood vessels to different levels in healthy teeth and teeth affected by caries. TLR4 mRNA, TLR4 protein, and mRNA of cytokine levels can be elevated with stimulations of LPS and extracts from S. mutans. Lipopolysaccharide and extracts from S. mutans treatment inhibited the proliferation of DPSCs but promoted migration; however, these changes were abolished when TLR4 was blocked by anti-TLR4 antibody. Conclusions: These results suggest that TLR4 will be activated and

regulate the proliferation and migration of DPSCs in deep caries. TLR4 may play an important role in the immune response by DPSCs.

13. **Li Y , Wang H, Pei F, Chen Z, Zhang L. FoxO3a Regulates Inflammation-induced Autophagy in Odontoblasts. J Endod. 2018 May; 44 (5): 786-791.**

Introduction: FoxO3a is a member of FoxO transcription factor family that participates in the transcriptional regulation of autophagy. In this study we explored the anti-inflammatory function of FoxO3a-regulated autophagy in inflamed human dental pulp and lipopolysaccharide-treated mDPC6T cells. Methods: The expression of FoxO3a and autophagy markers in caries and pulpitis from human dental pulp were examined by immunohistochemistry and Western blot. We conducted in vitro studies by treating mDPC6T cells with lipopolysaccharide for various lengths of time. Next, we measured the nuclear translocation of FoxO3a by immunofluorescence and investigated the potential relationship between FoxO3a and autophagy after FoxO3a knockdown using small interfering RNA. Results: The expression of FoxO3a and autophagy proteins was upregulated in the odontoblasts of human caries and pulpitis samples. In addition, we also observed that the enhanced nuclear translocation of FoxO3a was positively correlated with the progression of inflammation. The results of our in vitro study revealed that 6 hours of lipopolysaccharide treatment increased nuclear translocation of FoxO3a and activated autophagy in mDPC6T cells. We also observed that the knockdown of FoxO3a suppressed autophagy. Conclusions: Our data indicate that FoxO3a might play a role in autophagy activation and the maintenance of intracellular homeostasis in inflamed odontoblasts.

14. **Yumoto H, Hirao K, Hosokawa Y, Kuramoto H, Takegawa D, Nakanishi T. The roles of odontoblasts in dental pulp innate immunity. JADS. 2018 Mar; 194.**

Odontoblasts located in the outermost layer of dental pulp form a natural barrier between mineralized tissues, dentin, and soft tissues, dental pulp, of the vital tooth, and they first recognize caries-related pathogens and sense external irritations. Therefore, odontoblasts possess a specialized innate immune system to fight oral pathogens invading into dentin. Generally, the rapid initial sensing of microbial pathogens, especially pathogen-associated molecular patterns (PAMPs) shared by microorganisms, are mediated by pattern recognition receptors (PRRs), such as Toll-like receptor and the nucleotide-binding oligomerization domain (NOD). The innate immune responses in odontoblasts initiated by sensing oral pathogens provide host protective events, such as inflammatory reactions, to produce a variety of pro-inflammatory mediators, including chemokines and cytokines. These attract various inflammatory cells and cause antibacterial reactions, such as the production of defensins, to kill microorganisms in the proximal region of the odontoblast layer. This review focuses on innate immunity, especially cellular and molecular mechanisms regarding the sensing of PAMPs from oral pathogens by PRRs, in odontoblasts and provides information for future studies for the development of novel therapeutic strategies, including diagnosis and treatment, to prevent exceeding dental pulp inflammation and preserve the dental pulp tissues.

15. **Zhang L , Chen Z . Autophagy in the dentin-pulp complex against inflammation. Oral Dis. 2018 Mar; 24(1-2):11-13.**

The dentin-pulp complex is a highly specialized tissue for protecting the dental pulp. Odontoblasts are long-lived, hard-tissue-forming cells in the dentin-pulp complex and critically involved in inflammatory responses against invading

pathogens. Autophagy is a highly conserved homeostasis mechanism of living cells under various stress conditions. Growing evidence in the literature addresses the role of autophagy in odontoblast differentiation and aging. This review summarizes the current knowledge about autophagy for the dentin-pulp complex in resisting inflammation.

16. **Takanchea J, Kima J-S, Kima J-E, Hanb S-H, Yia H-K. Schisandrin C enhances odontoblastic differentiation through autophagy and mitochondrial biogenesis in human dental pulp cells. Archives of Oral Biology. 2018 Jan. 88:60–66.**

Objective: To investigate the role of Schisandrin C in odontoblastic differentiation, and its relations between autophagy and mitochondrial biogenesis in human dental pulp cells (HPDCs). Design: Fresh third molars were used, and cultured for HDPCs. Western blotting technique, Alizarin red S staining, alkaline phosphatase (ALP) activity, and confocal microscopy were used to detect autophagy, mitocondrial biogenesis, and odontoblastic differentiation. To understand the mechanism of Schisandrin C, the HDPCs were treated with lipopolysaccharide (LPS), autophagy and heme oxygenase-1 (HO-1) inhibitors: 3- Methyladenine (3-MA) and Zinc protoporphyrin IX (ZnPP), respectively. Results: LPS decreased the expression of autophagy molecules [autophagy protein 5 (ATG-5), beclin-1, and microtubule-associated protein 1A/1B light chain 3 (LC3-I/II)] and mitochondrial biogenesis molecules [heme oxygenase-1 (HO-1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)], and disrupted odontoblastic differentiation. The down-regulation of autophagy and mitochondrial biogenesis with 3- MA and ZnPP inhibited odontoblastic differentiation. However, Schisandrin C restored the expression of all the above molecules, even with LPS and inhibitor treatment. This result demonstrates that autophagy and mitochondrial biogenesis plays an essential role in odontoblastic differentiation, and Schisandrin C activates these systems to promote odontoblastic differentiation of HDPCs. Conclusion: Schisandrin C has potential characters to regulate odontoblastic differentiation, and may be recommended for use as a compound for pulp homeostasis.

17. **Keller JF, Carrouel F, Colomb E, Durand SH, Baudouin C, Msika P, Bleicher F, Vincent C, Staquet M.J, Farges J.C. Toll-like receptor 2 activation by lipoteichoic acid induces differential production of pro-inflammatory cytokines in human odontoblasts, dental pulp fibroblasts and immature dendritic cells. Immunobiology. 2010 Jan. 215: 53-59.**

Odontoblasts, dental pulp fibroblasts and immature dendritic cells (DCs) have been involved in the human dental pulp immune response to oral pathogens that invade dentine during thecaries process. How they regulate the inflammatory response to Gram-positive bacteriaremains nevertheless largely unknown. In this study we investigated the production of the pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-8 (CXCL8) in these three cell types upon stimulation with lipoteichoic acid(LTA), a cell wall component of Gram-positive bacteria that activates the pattern recognition molecule Toll-like receptor 2 (TLR2). We observed that TNF-α gene expression was up-regulated in all LTA-stimulated cell types. IL-1β gene expression was not or barely detectable in odontoblast-like cells and pulp fibroblasts when stimulated or not, but was expressed in immature DCs and increased upon stimulation. TNF-α and IL-1β proteins were detected in DC culture supernatants but not in odontoblast-like cell and pulp fibroblast ones. CXCL8 gene and protein were clearly expressed and increased in the three cell types upon LTA stimulation. These data indicate that LTA-dependent TLR2 activation in odontoblasts and pulp fibroblasts, in contrast to immature DCs, does

not lead to significant TNF-α and IL-1β production, but that all three cell types influence the pulp inflammatory/immune response through CXCL8 synthesis and secretion.

18. **Kim JH, Woo SM, Choi NK, Kim WJ, Kim SM, Jung JY. Effect of Platelet-rich Fibrin on Odontoblastic Differentiation in Human Dental Pulp Cells Exposedto Lipopolysaccharide. J Endod. 2017 Mar;43(3):433-438.**

Introduction: Platelet-rich fibrin (PRF), as an autologous fibrin matrix, is known to contain platelets, leukocytes, and growth factors to control inflammation and to facilitate the healing process. The purpose of this study was to investigate the effects of PRF on odontoblasticdifferentiation in human dental pulp cells (HDPCs) treated with lipopolysaccharide (LPS). Methods: Gene expression of inflammatory cytokines and adhesion molecules on the HDPCs cultured with or without LPS and PRF extract (PRFe) were evaluated by reverse-transcription polymerase chain reaction and Western blot analysis. In addition, odontoblastic differentiationwas determined by measuring alkaline phosphatase (ALP) activity using ALP staining, the expression of odontogenesis-related genes, and the extent of mineralization using alizarin red S staining. Results: Treatment with PRFe significantly attenuated the LPS-stimulated expression of interleukin (IL)-1β, IL-6, and IL-8 in HDPCs. In addition, PRFe inhibited the up-regulation of vascular cell adhesion molecule 1 and the production of intracellular adhesion molecule 1 in HDPCs exposed to LPS. Expression of dentin sialophosphoprotein and dentin matrix acidic phosphoprotein 1, ALP activity, and mineralization were enhanced by PRFe in LPS-treated HDPCs. Conclusions: These results suggest that PRF has effects associated not only with inhibition of inflammation in HDPCs exposed to LPS but also stimulation of odontoblastic differentiation.

19. **Farges J-C, Carrouwl F, Keller J-F, Baudouin C, Msika P, Bleicher F, Staquet M-J. Cytokine production by human odontoblast-like cells upon Toll-like receptor-2 engagement. Immunobiology, 2011 Apr; 216 (4): 513-517.**

Recent studies have suggested that odontoblasts are involved in the dental pulp immune response to oral pathogens that invade human dentin during the caries process. How odontoblasts regulate the early inflammatory and immune pulp response to Gram-positive bacteria, which predominate in shallow and moderate dentin caries, is still poorly understood. In this study, we investigated the production of pro- and anti-inflammatory cytokines by odontoblast-like cells upon engagement of Toll-like receptor (TLR) 2, a pattern recognition molecule activated by Gram-positive bacteria components. We used a highly sensitive Milliplex® kit for detecting cytokines released by cells stimulated with lipoteichoic acid (LTA), a cell wall component of Gram-positive bacteria, or with the potent TLR2 synthetic agonist Pam2CSK4. We found that odontoblasts produce the pro-inflammatory cytokines interleukin (IL)-6 and CXCL8, as well as the immunosuppressive cytokine IL-10 in response to TLR2 agonists. GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-12(p70), IL-13and TNF-α were not detected. These data indicate that TLR2 activation in human odontoblasts selectively induces production of mediators known to influence positively or negatively inflammatory and immune responses in pathogen-challenged tissues. We suggest that these molecules might be important in regulating the fine tuning of the pulp response to Gram-positive bacteria which enter dentin during the caries process.

20. **Horst OV, Horst JA, Samudrala R, Dale BA. Caries induced cytokine network in the odontoblast layer of human teeth. BMC Immunol. 2011 Jan; 24;12:9.**

Background: Immunologic responses of the tooth to caries begin with odontoblasts recognizing carious bacteria. Inflammatory propagation eventually leads to tooth pulp necrosis and danger to health. The present study aims to determine cytokine gene expression profiles generated within human teeth in response to dental caries in vivo and to build a mechanistic model of these responses and the downstream signaling network. Results: We demonstrate profound differential up-regulation of inflammatory genes in the odontoblast layer (ODL) in human teeth with caries in vivo, while the pulp remains largely unchanged. Interleukins, chemokines, and all tested receptors thereof were differentially up-regulated in ODL of carious teeth, well over one hundred-fold for 35 of 84 genes. By interrogating reconstructed protein interaction networks corresponding to the differentially up-regulated genes, we develop the hypothesis that pro-inflammatory cytokines highly expressed in ODL of carious teeth, IL-1β, IL-1α, and TNF-α, carry the converged inflammatory signal. We show that IL1β amplifies antimicrobial peptide production in odontoblasts in vitro 100-fold more than lipopolysaccharide, in a manner matching subsequent in vivo measurements. Conclusions: Our data suggest that ODL amplifies bacterial signals dramatically by self-feedback cytokine-chemokine signal-receptor cycling, and signal convergence through IL1R1 and possibly others, to increase defensive capacity including antimicrobial peptide production to protect the tooth and contain the battle against carious bacteria within the dentin.

**21. Song Z, Lin Z, He F, Jiang L, Qin W, Tian Y, Wang R, Huan. NLRP3 is expressed in human dental pulp cells and tissues. J Endod. 2012 Dec;38(12):1592-7**

Introduction: One of the best-characterized Nod-like receptor (NLR) family members is pyrin domain containing 3 (NLRP3). Intracellular NLRP3 is the most versatile innate immune receptor. On activation, NLRP3 assembles into a multiprotein complex, termed an inflammasome, which regulates the secretion and bioactivity of interleukin-1 family cytokines. NLRP3 has broad specificity for mediating an immune response to a wide range of microbial stimuli or danger signals. Therefore, we hypothesize that NLRP3 plays an essential role in the detection of bacterial pathogens and the initiation of inflammation within the dental pulp. Thus, the aim of this study was to evaluate the expression of NLRP3 in normal human dental pulp cells (HDPCs) and pulp tissues. Methods: Pulp tissues were collected from freshly extracted human third molars, and HDPCs were prepared from the explants of normal dental pulp tissues. Reverse transcription-polymerase chain reaction and Western blotting were performed to detect the levels of NLRP3mRNA and protein, respectively. In addition, immunohistochemical staining was used to determine the distribution of NLRP3 in pulp tissues. Results: Normal human dental pulp tissues displayed high levels of NLRP3 mRNA and protein. NLRP3 proteins were principally expressed in odontoblasts and some pulp vascular endothelial cells. Moreover, HDPCs also expressed NLRP3 but at a relatively low level in comparison with that of dental pulp tissues. Conclusions: The expression of NLRP3 in HDPCs and pulp tissues suggests that NLRP3-mediated signaling pathways may play an important role in dental immune defense.

**22. Yang CS, Shin DM, Jo EK. The Role of NLR-related Protein 3 Inflammasome in Host Defense and Inflammatory Diseases. Int Neurourol J. 2012 Mar;16(1): 2-12.**

Among a number of innate receptors, the nucleotide-binding domain leucine-rich repeat containing (NLR) nucleotide oligomerization domain (NOD)-like receptor families are involved in the recognition of cytosolic pathogen- or danger-associated molecules. Activation of these specific sets of receptors leads to the assembly of a multiprotein complex, the inflammasome, leading to the activation of caspase-1 and maturation of the cytokines interleukin (IL)-1β, IL-18, and IL-

33. Among NLRs, NLR-related protein 3 (NLRP3) is one of the best-characterized receptors that activates the inflammasome. There is no doubt that NLRP3 inflammasome activation is important for hostdefense and effective pathogen clearance against fungal, bacterial, and viral infection. In addition, mounting evidence indicates that the NLRP3 inflammasome plays a role in a variety of inflammatory diseases, including gout, atherosclerosis, and type II diabetes, as well as under conditions of cellular stress or injury. Here, we review recent advances in our understanding of the role of the NLRP3 inflammasome in host defense and various inflammatory diseases.

**23. Pei F, Lin H, Liu H, Li L, Zhang L, Chen Z. Dual role of autophagy in lipopolysaccharide-induced preodontoblastic cells. J Dent Res. 2015 Jan;94(1):175-82.**

Odontoblasts derive from neural crest-derived odontogenic mesenchymal cells, and they are an important barrier of defense for the host. Survival and immunity of odontoblasts play important roles in protecting the dentin-pulp structure. Autophagy can eliminate damaged organelles and recycle cellular components to facilitate cellular homeostasis. Autophagy can be activated with external stressors, such as starvation, hypoxia, and infection. In this study, the role of autophagy in inflamed odontoblasts was explored, and its possible mechanism was investigated. Cell viability was not affected by mild lipopolysaccharide (LPS) stimulation, and autophagy was activated during this process. Immunofluorescence of light chain 3 confirmed that autophagy was induced with LPS treatment. Early-stage autophagy inhibition resulted in down-regulated cell viability, contrary to the up-regulated cell viability at late-stage autophagy inhibition. Western blot suggested that p-Akt and survivin were not activated in the early stage, and they gradually increased and peaked in the late stage. Meanwhile, autophagy was down-regulated through the Akt/mTOR/survivin pathway in the late stage. Thus, autophagy has a dual role in inflamed odontoblasts, which indicates its importance in maintaining the microenvironment homeostasis of odontoblasts. Autophagy was induced as a survival mechanism in the early stage, and it decreased through the Akt/mTOR/survivin signaling pathway in the late stage.

**24. Pei F, Wang HS, Chen Z, Zhang L. Autophagy regulates odontoblast differentiation by suppressing NF-κB activation in an inflammatory environment. Cell Death Dis. 2016 Mar 3;7:e2122.**

Odontoblasts are derived from dental papilla mesenchymal cells and have an important role in defense against bacterial infection, whereas autophagy can recycle long-lived proteins and damaged organelles to sustain cellular homeostasis. Thus, this study explores the role of autophagy in odontoblast differentiation with lipopolysaccharide (LPS) stimulation in vitro and the colocalization of p-NF-κB and LC3 in caries teeth. The odontoblasts differentiation was enhanced through LPS stimulation, and this outcome was reflected in the increased number of mineralized nodules and alkaline phosphatase (ALP) activity. The expression levels of the autophagy markers LC3, Atg5, Beclin1 and TFE3 increased time dependently, as well along with the amount of autophagosomes and autophagy fluxes. This result suggests that autophagywas enhanced in odontoblasts cultured with mineralized-induced media containing LPS. To confirm the role of autophagy in differentiated odontoblasts with LPS stimulation, chloroquine (CQ) or rapamycin were used to either block or enhance autophagy. The number of mineralized nodules decreased when autophagy was inhibited, but this number increased with rapamycin treatment. Phosphorylated nuclear factor-κB (NF-κB) expression was negatively related to autophagy and could inhibit odontoblast differentiation. Furthermore, p-NF-κB and LC3 colocalization could be detected in cells stimulated with LPS. The nucleus translocation of p-NF-κB in odontoblasts was enhanced when autophagy was inhibited by Atg5 small interfering RNA. In addition, the colocalization of p-NF-κB and LC3 in odontoblasts and sub-odontoblastic layers was

observed in caries teeth with reactionary dentin. Therefore, our findings provide a novel insight into the role of autophagy in regulating odontoblast differentiation by suppressing NF-κB activation in inflammatory environments.

**25. Ikeda E, Goto T, Gunjigake K, Kuroishi K, Ueda M, Kataoka S, Toyono T, Nakatomi M, Seta Y, Kitamura C, Nishihara T, Kawamoto T. Expression of Vesicular Nucleotide Transporter in Rat Odontoblasts. Acta Histochem Cytochem. 2016 Feb 27;49(1):21-8.**

Several theories have been proposed regarding pain transmission mechanisms in tooth. However, the exact signaling mechanism from odontoblasts to pulp nerves remains to be clarified. Recently, ATP-associated pain transmission has been reported, but it is unclear whether ATP is involved in tooth pain transmission. In the present study, we focused on the vesicular nucleotide transporter (VNUT), a transporter of ATP into vesicles, and examined whether VNUT was involved in ATP release from odontoblasts. We examined the expression of VNUT in ratpulp by RT-PCR and immunostaining. ATP release from cultured odontoblast-like cells with heat stimulation was evaluated using ATP luciferase methods. VNUT was expressed in pulp tissue, and the distribution of VNUT-immunopositive vesicles was confirmed in odontoblasts. In odontoblasts, some VNUT-immunopositive vesicles were colocalized with membrane fusion proteins. Additionally P2X3, an ATP receptor, immunopositive axons were distributed between odontoblasts. The ATP release by thermal stimulation from odontoblast-like cells was inhibited by the addition of siRNA for VNUT. These findings suggest that cytosolic ATP is transported by VNUT and that the ATP in the vesicles is then released from odontoblasts to ATP receptors on axons. ATP vesicle transport in odontoblasts seems to be a key mechanism for signal transduction from odontoblasts to axons in the pulp.

**26. Pääkkönen V, Rusanen P, Hagström J, Tjäderhane L. Mature human odontoblasts express virus-recognizing toll-like receptors. Int Endod J. 2014 Oct;47(10):934-41.**

Aim: To study the expression of toll-like receptors (TLR) -3, -7, -8 and -9 as well as interferon receptors alpha and gamma (IFNAR1/IFNAR2 and IFNGR1/IFNGR2), which play important roles in the defence against viruses. Methodology: DNA microarray and quantitative PCR analyses of TLR3, -7, -8 and -9 as well as IFNAR1/IFNAR2 and IFNGR1/IFNGR2 genes in mature native human odontoblasts and pulp were performed. Immunohistochemistry was used to confirm TLR8 protein in odontoblasts of healthy and carious human teeth. Results: TLR3, -7, -8 and -9 mRNAs were detected both in odontoblasts and in pulp, but TLR8 expression level was higher in the odontoblasts. IFNAR and IFNGR expression was observed in both tissues. Immunohistochemical analysis of healthy teeth revealed positive TLR8 staining in the pre-dentine and the dentine but varying staining patterns in the different portions of tooth. Lighter TLR8 staining was observed in dentine of mildly carious teeth. In teeth with carious lesions extending into the mid-dentine, only very weak staining was detected. Conclusions: The finding of these virus-recognition-related genes in odontoblasts strengthens the view that odontoblasts participate in the immune response of the dentine-pulp complex.

**27. He W, Wang Z, Luo Z, Yu Q, Jiang Y, Zhang Y, Zhou Z, Smith AJ, Cooper PR. LPS promote the odontoblastic differentiation of human dental pulp stem cells via MAPK signaling pathway. J Cell Physiol. 2015 Mar;230(3):554-61.**

Human dental pulp stem cells (hDPSCs) show significant potential for exploitation in novel regeneration strategies, although lack of understanding of their responses to bacterial challenge constrains their application. The present study

aimed to investigate whether lipopolysaccharide (LPS), the major pathogenic factor of Gram-negative bacteria, regulates the differentiation of hDPSCs and which intracellular signaling pathways may be involved. LPS treatment significantly promoted the differentiation of hDPSCs demonstrable by increased mineralized nodule formation and mRNA expression of several odontoblastic markers in a dose-dependent manner. While inhibition of TLR4, p38, and ERK signaling markedly antagonized LPS-mediated differentiation of hDPSCs. The inhibition of JNK and NF-κB signaling had no detectable effect on LPS activation of hDPSCs. LPS stimulation resulted in phosphorylation of NF-κB p65, IκB-α, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) in DPSCs in a time-dependent manner, which was markedly suppressed by their specific inhibitors, respectively. Data demonstrated that LPS promoted odontoblastic differentiation of hDPSCs via TLR4, ERK, and P38 MAPK signaling pathways, but not NF-κB signaling.

**28. Zhang J, Zhu QL, Huang P, Yu Q, Wang ZH, Cooper PR, Smith AJ, He W. CpG ODN-induced matrix metalloproteinase-13 expression is mediated via activation of the ERKand NF-κB signalling pathways in odontoblast cells. Int Endod J. 2013 Jul;46(7):666-74.**

Aim: To investigate the effects of CpG ODN (CpG oligodeoxynucleotides) to model the action of bacterial challenge on pulpal matrix metalloproteinase-13 (MMP-13) expression and elucidate the associated intracellular signalling pathways. Methodology: Real-time PCR was used to detect the effects of CpG ODN on MMP-13 mRNA expression levels in a murine odontoblast-lineage cell line (OLCs). The possible involvement of TLR9/MyD88, NF-κB or MAPK pathways involved in the CpG ODN-induced MMP-13 expression was examined by real-time PCR, transient transfection, luciferase activity assay and ELISA. Western blotting was performed to assay the phosphorylation of ERK at a range of time points. Results: MMP-13 was constitutively expressed in OLCs, and their exposure to CpG ODN significantly increased MMP-13 expression. Pre-treatment of OLCs with the inhibitory peptide MyD88, or chloroquine, attenuated the CpG ODN-induced expression of MMP-13. Treatment of the OLCs with CpG ODN increased NF-κB-luciferase activity. This activity was decreased by the over-expression of a nondegrading mutant of IκBα (IκBαSR), although enhanced by the over-expression of NF-κB p65. MMP-13 expression induced by CpG ODN was markedly suppressed by NF-κB inhibitors (pyrrolidine dithiocarbamate, PDTC), IκBα phosphorylation inhibitors (Bay 117082) or IκB protease inhibitor (L-1-tosylamido-2-phenylethyl chloromethyl ketone, TPCK). The inhibitor of ERK1/2, U0126, but not inhibitors of p38 MAPK and JNK, SB203580 and SP600125, decreased CpG ODN-mediated MMP-13 expression. Conclusion: The CpG ODN-induced MMP-13 expression in OLCs is mediated through TLR9, NF-κB and the ERK pathway indicating that potentially the recognition of CpG ODN by TLR9 on odontoblasts may regulate the remodelling of injured dental pulp and hard tissues by inducing MMP-13 expression.

**29. Utreras E, Prochazkova M, Terse A, Gross J, Keller J, Iadarola MJ, Kulkarni AB. TGF-β1 sensitizes TRPV1 through Cdk5 signaling in odontoblast-like cells. Mol Pain. 2013 May; 13;9:24.**

Background: Odontoblasts are specialized cells that form dentin and they are believed to be sensors for tooth pain. Transforming growth factor-β1 (TGF-β1), a pro-inflammatory cytokine expressed early in odontoblasts, plays an important role in the immune response during tooth inflammation and infection. TGF-β1 is also known to participate in pain signaling by regulating cyclin-dependent kinase 5 (Cdk5) in nociceptive neurons of the trigeminal and dorsal root ganglia. However, the precise role of TGF-β1 in tooth pain signaling is not well characterized. The aim of our present study was to determine whether or not in odontoblasts Cdk5 is functionally active, if it is regulated by TGF-β1, and if it affects

the downstream pain receptor, transient receptor potential vanilloid-1 (TRPV1). Results: We first determined that Cdk5 and p35 are indeed expressed in an odontoblast-enriched primary preparation from murine teeth. For the subsequent analysis, we used an odontoblast-like cell line (MDPC-23) and found that Cdk5 is functionally active in these cells and its kinase activity is upregulated during cell differentiation. We found that TGF-β1 treatment potentiated Cdk5 kinase activity in undifferentiated MDPC-23 cells. SB431542, a specific inhibitor of TGF-β1 receptor 1 (Tgfbr1), when co-administered with TGF-β1, blocked the induction of Cdk5 activity. TGF-β1 treatment also activated the ERK1/2 signaling pathway, causing an increase in early growth response-1 (Egr-1), a transcription factor that induces p35 expression. In MDPC-23 cells transfected with TRPV1, Cdk5-mediated phosphorylation of TRPV1 at threonine-407 was significantly increased after TGF-β1 treatment. In contrast, SB431542 co-treatment blocked TRPV1 phosphorylation. Moreover, TGF-β1 treatment enhanced both proton- and capsaicin-induced $Ca^{2+}$ influx in TRPV1-expressing MDPC-23 cells, while co-treatment with either SB431542 or roscovitine blocked this effect. Conclusions: Cdk5 and p35 are expressed in a murine odontoblast-enriched primary preparation of cells from teeth. Cdk5 is also functionally active in odontoblast-like MDPC-23 cells. TGF-β1 sensitizes TRPV1 through Cdk5 signaling in MDPC-23 cells, suggesting the direct involvement of odontoblasts and Cdk5 in dental nociceptive pain transduction.

**30. Couve E, Osorio R, Schmachtenberg O. Mitochondrial autophagy and lipofuscin accumulation in aging odontoblasts. J Dent Res. 2012 Jul;91(7):696-701.**

Aging of long-lived post-mitotic cells is characterized as a progressive and irreversible reduction of functional activity. In such cells, mitochondria are organelles critical for bioenergetic supply, whose turnover is mediated by an autophagic-lysosomal pathway. In human teeth, odontoblasts are post-mitotic cells responsible for sensory function and dentin preservation. Here, human odontoblasts were processed for immunohistochemistry with antibodies against mitochondrial (MTCO2) and lysosomal (LAMP2) markers, and comparatively analyzed in two age groups (young-adult and adult) with light and electron microscopy. Selective engulfment of mitochondrial profiles into autophagic vacuoles is common in young-adult odontoblasts, suggesting a microautophagic pathway. With age, the odontoblast layer is reduced in width, and mitochondrial elements converge around large clusters of autofluorescent lipofuscin deposits. Age-related changes in odontoblasts are observed as a long-term process in which the progressive accumulation of intralysosomal debris limits the autophagic turnover of mitochondrial components, causing an eventual decline in physiological cell functions, which leads to increased vulnerability under stress conditions.

**31. Couve E, Schmachtenberg O. Autophagic activity and aging in human odontoblasts. J Dent Res. 2011 Apr;90(4):523-8.**

Odontoblasts are long-lived post-mitotic cells in the dental pulp, whose function is to form and maintain dentin. The survival mechanisms that preserve the viability of terminally differentiated odontoblasts during the life of a healthy tooth have not been described. In the present study, we characterized the autophagic-lysosomal system of human odontoblasts with transmission electron microscopy and immunocytochemistry, to analyze the mechanisms that maintain the functional viability of these dentinogenic cells. Odontoblasts were found to develop an autophagic-lysosomal system organized mainly by large autophagic vacuoles that are acid-phosphatase-positive to various degrees. These vacuoles expressed the autophagosomal and lysosomal markers LC3 and LAMP2, respectively, in an age-related pattern indicating the organization

of a dynamic autophagic machinery. Progressive accumulation of lipofuscin within lysosomes indicates reduced lysosomal activity as a function of odontoblast aging. Our results suggest that autophagic activity in odontoblasts is a fundamental mechanism to ensure turnover and degradation of subcellular components. A reduction in the efficacy of this system might compromise cell viability and dentinogenic secretory capacity. In adult teeth, this condition is described as an 'old odontoblast' stage.

**32. Song F, Sun H, Wang Y, Yang H, Huang L, Fu D, Gan J, Huang C. Pannexin3 inhibits TNF-α-induced inflammatory response by suppressing NF-κB signalling pathway in human dental pulp cells. J Cell Mol Med. 2017 Mar; 21(3):444-455.**

Human dental pulp cells (HDPCs) play a crucial role in dental pulp inflammation. Pannexin 3 (Panx3), a member of Panxs (Pannexins), has been recently found to be involved in inflammation. However, the mechanism of Panx3 in human dental pulp inflammation remains unclear. In this study, the role of Panx3 in inflammatory response was firstly explored, and its potential mechanism was proposed. Immunohistochemical staining showed that Panx3 levels were diminished in inflamed human and rat dental pulp tissues. In vitro, Panx3 expression was significantly down-regulated in HDPCs following a TNF-α challenge in a concentration-dependent way, which reached the lowest level at 10 ng/ml of TNF-α. Such decrease could be reversed by MG132, a proteasome inhibitor. Unlike MG132, BAY 11-7082, a NF-κB inhibitor, even reinforced the inhibitory effect of TNF-α. Quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) were used to investigate the role of Panx3 in inflammatory response of HDPCs. TNF-α-induced pro-inflammatory cytokines, interleukin (IL)-1β and IL-6, were significantly lessened when Panx3 was overexpressed in HDPCs. Conversely, Panx3 knockdown exacerbated the expression of pro-inflammatory cytokines. Moreover, Western blot, dual-luciferase reporter assay, immunofluorescence staining, qRT-PCR and ELISA results showed that Panx3 participated in dental pulp inflammation in a NF-κB-dependent manner. These findings suggested that Panx3 has a defensive role in dental pulp inflammation, serving as a potential target to be exploited for the intervention of human dental pulp inflammation.

**33. Iwamoto T , Nakamura T , Ishikawa M , Yoshizaki K , Sugimoto A , Ida-Yonemochi H , Ohshima H [6] , Saito M , Yamada Y , Fukumoto S. Pannexin 3 regulates proliferation and differentiation of odontoblasts via its hemichannelactivities. PLoS One. 2017 May 11;12(5):e0177557.**

Highly coordinated regulation of cell proliferation and differentiation contributes to the formation of functionally shaped and sized teeth; however, the mechanism underlying the switch from cell cycle exit to cell differentiation during odontogenesis is poorly understood. Recently, we identified pannexin 3 (Panx3) as a member of the pannexin gap junction protein family from tooth germs. The expression of Panx3 was predominately localized in preodontoblasts that arise from dental papilla cells and can differentiate into dentin-secreting odontoblasts. Panx3 also co-localized with p21, a cyclin-dependent kinase inhibitor protein, in preodontoblasts. Panx3 was expressed in primary dental mesenchymal cells and in the mDP dental mesenchymal cell line. Both Panx3 and p21 were induced during the differentiation of mDP cells. Overexpression of Panx3 in mDP cells reduced cell proliferation via up-regulation of p21, but not of p27, and promoted the Bone morphogenetic protein 2 (BMP2)-induced phosphorylation of Smad1/5/8 and the expression of dentin sialophosphoprotein (Dspp), a marker of differentiated odontoblasts. Furthermore, Panx3 released intracellular ATP into the extracellular space through its hemichannel and induced the phosphorylation of AMP-activated protein kinase (AMPK). 5-Aminoimidazole-4-

carboxamide-ribonucleoside (AICAR), an activator of AMPK, reduced mDP cell proliferation and induced p21 expression. Conversely, knockdown of endogenous Panx3 by siRNA inhibited AMPK phosphorylation, p21 expression, and the phosphorylation of Smad1/5/8 even in the presence of BMP2. Taken together, our results suggest that Panx3 modulates intracellular ATP levels, resulting in the inhibition of odontoblast proliferation through the AMPK/p21 signaling pathway and promotion of cell differentiation by the BMP/Smad signaling pathway.

**34. Shibukawa Y, Sato M, Kimura M, Sobhan U, Shimada M, Nishiyama A, Kawaguchi A, Soya M, Kuroda H, Katakura A, Ichinohe T, Tazaki M. Odontoblasts as sensory receptors: transient receptor potential channels, pannexin-1, and ionotropic ATP receptors mediate intercellular odontoblast-neuron signal transduction. Pflugers Arch. 2015 Apr;467(4):843-63.**

Various stimuli induce pain when applied to the surface of exposed dentin. However, the mechanisms underlying dentinal pain remain unclear. We investigated intercellular signal transduction between odontoblasts and trigeminal ganglion (TG) neurons following direct mechanical stimulation of odontoblasts. Mechanical stimulation of single odontoblasts increased the intracellular free calcium concentration ([Ca(2+)]i) by activating the mechanosensitive-transient receptor potential (TRP) channels TRPV1, TRPV2, TRPV4, and TRPA1, but not TRPM8 channels. In cocultures of odontoblasts and TG neurons, increases in [Ca(2+)]i were observed not only in mechanically stimulated odontoblasts, but also in neighboring odontoblasts and TG neurons. These increases in [Ca(2+)]i were abolished in the absence of extracellular Ca(2+) and in the presence of mechanosensitive TRP channel antagonists. A pannexin-1 (ATP-permeable channel) inhibitor and ATP-degrading enzyme abolished the increases in [Ca(2+)]i in neighboring odontoblasts and TG neurons, but not in the stimulated odontoblasts. G-protein-coupled P2Y nucleotide receptor antagonists also inhibited the increases in [Ca(2+)]i. An ionotropic ATP (P2X3) receptor antagonist inhibited the increase in [Ca(2+)]i in neighboring TG neurons, but not in stimulated or neighboring odontoblasts. During mechanical stimulation of single odontoblasts, a connexin-43 blocker did not have any effects on the [Ca(2+)]i responses observed in any of the cells. These results indicate that ATP, released from mechanically stimulated odontoblasts via pannexin-1 in response to TRP channel activation, transmits a signal to P2X3 receptors on TG neurons. We suggest that odontoblasts are sensory receptor cells and that ATPreleased from odontoblasts functions as a neurotransmitter in the sensory transduction sequence for dentinal pain.

**35. Bond SR, Naus CC. The pannexins: past and present. Front Physiol. 2014 Feb 19;5:58.**

The pannexins (Panxs) are a family of chordate proteins homologous to the invertebrate gap junction forming proteins named innexins. Three distinct Panx paralogs (Panx1, Panx2, and Panx3) are shared among the major vertebrate phyla, but they appear to have suppressed (or even lost) their ability to directly couple adjacent cells. Connecting the intracellular and extracellular compartments is now widely accepted as Panx's primary function, facilitating the passive movement of ions and small molecules along electrochemical gradients. The tissue distribution of the Panxs ranges from pervasive to very restricted, depending on the paralog, and are often cell type-specific and/or developmentally regulated within any given tissue. In recent years, Panxs have been implicated in an assortment of physiological andpathophysiological processes, particularly with respect to ATP signaling and inflammation, and they are now considered to be a major player in extracellular purinergic communication. The following is a comprehensive review of

the Panx literature, exploring the historical events leading up to their discovery, outlining our current understanding of their biochemistry, and describing the importance of these proteins in health and disease.

**36. Tsumura M, Sobhan U, Sato M, Shimada M, Nishiyama A, Kawaguchi A, Soya M, Kuroda H, Tazaki M, Shibukawa Y. Functional expression of TRPM8 and TRPA1 channels in rat odontoblasts. PLoS One. 2013 Dec 16;8(12):e82233.**

Odontoblasts produce dentin during development, throughout life, and in response to pathological conditions by sensing stimulation of exposed dentin. The functional properties and localization patterns of transient receptor potential (TRP) melastatin subfamily member 8 (TRPM8) and ankyrin subfamily member 1 (TRPA1) channels in odontoblasts remain to be clarified. We investigated the localization and the pharmacological, biophysical, and mechano-sensitive properties of TRPM8 and TRPA1 channels in rat odontoblasts. Menthol and icilin increased the intracellular free Ca(2+) concentration ([Ca(2+)]i). Icilin-, WS3-, or WS12-induced [Ca(2+)]i increases were inhibited by capsazepine or 5-benzyloxytriptamine. The increase in [Ca(2+)]i elicited by allyl isothiocyanate (AITC) was inhibited by HC030031. WS12 and AITC exerted a desensitizing effect on [Ca(2+)]i increase. Low-temperature stimuli elicited [Ca(2+)]i increases that are sensitive to both 5-benzyloxytriptamine and HC030031. Hypotonic stimulation-induced membrane stretch increased [Ca(2+)]i; HC030031 but not 5-benzyloxytriptamine inhibited the effect. The results suggest that TRPM8 channels in rat odontoblasts play a role in detecting low-temperature stimulation of the dentin surface and that TRPA1 channels are involved in sensing membrane stretching and low-temperature stimulation. The results also indicate that odontoblasts act as mechanical and thermal receptor cells, detecting the stimulation of exposed dentin to drive multiple cellular functions, such as sensory transduction.

**37. Wetsel WC. Sensing hot and cold with TRP channels. Int J Hyperthermia. 2011;27(4):388-98.**

The past decade has witnessed the cloning of a new family of ion channels that are responsive to temperature. Six of these transient receptor potential (TRP) channels are proposed to be involved in thermosensation and are located in sensory nerves and skin. The TRPV1, TRPV2, TRPV3, and TRPV4 channels have incompletely overlapping functions over a broad thermal range from warm to hot. Deletion of the individual TRPV1, TRPV3, and TRPV4 channels in mice has established their physiological role in thermosensation. In all cases thermosensation is not completely abolished - suggesting some functional redundancy among the channels. Notably, the TRPV2 channel is responsive to hot temperatures in heterologous systems, but its physiological relevance in vivo has not been established. Cool and cold temperatures are sensed by TRPM8 and TRPA1 family members. Currently, the pharmaceutical industry is developing agonists and antagonists for the various TRP channels. For instance, TRPV1 receptor agonists produce hypothermia, while antagonists induce hyperthermia. Recent investigations have found that different regions of the TRPV1 receptor are responsive to temperature, nociceptive stimuli, and various chemical agents. With this information, it has been possible to develop a TRPV1 compound that blocks responses to capsaicin and acid while leaving temperature sensitivity intact. These channels have important implications for hyperthermia research and may help to identify previously unexplored mechanisms in different tissues that are responsive to thermal stress.

**38. Jardín I, López JJ, Diez R, Sánchez-Collado J, Cantonero C, Albarrán L, Woodard GE, Redondo PC, Salido GM, Smani T, Rosado JA. TRPs in Pain Sensation. Front Physiol. 2017 Jun 9;8:392.**

According to the International Association for the Study of Pain (IASP) pain is characterized as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage". The TRP super-family, compressing up to 28 isoforms in mammals, mediates a myriad of physiological and pathophysiological processes, pain among them. TRP channel might be constituted by similar or different TRP subunits, which will result in the formation of homomeric or heteromeric channels with distinct properties and functions. In this review we will discuss about the function of TRPs in pain, focusing on TRP channles that participate in the transduction of noxious sensation, especially TRPV1 and TRPA1, their expression in nociceptors and their sensitivity to a large number of physical and chemical stimuli.

**39. Sato M, Furuya T, Kimura M, Kojima Y, Tazaki M, Sato T, Shibukawa Y. Intercellular Odontoblast Communication via ATP Mediated by Pannexin-1 Channel and Phospholipase C-coupled Receptor Activation. Front Physiol. 2015 Nov 10;6:326.**

Extracellular ATP released via pannexin-1 channels, in response to the activation of mechanosensitive-TRP channels during odontoblast mechanical stimulation, mediates intercellular communication among odontoblasts in dental pulp slice preparation dissected from rat incisor. Recently, odontoblast cell lines, such as mouse odontoblast lineage cells, have been widely used to investigate physiological/pathological cellular functions. To clarify whether the odontoblast cell lines also communicate with each other by diffusible chemical substance(s), we investigated the chemical intercellular communication among cells from mouse odontoblast cell lines following mechanical stimulation. A single cell was stimulated using a glass pipette filled with standard extracellular solution. We measured intracellular free Ca(2+) concentration ([Ca(2+)]i) by fura-2 in stimulated cells, as well as in cells located nearby. Direct mechanical stimulation to a single odontoblast increased [Ca(2+)]i, which showed sensitivity to capsazepine. In addition, we observed increases in [Ca(2+)]i not only in the mechanically stimulated odontoblast, but also in nearby odontoblasts. We could observe mechanical stimulation-induced increase in [Ca(2+)]i in a stimulated human embryo kidney (HEK) 293 cell, but not in nearby HEK293 cells. The increase in [Ca(2+)]i in nearby odontoblasts, but not in the stimulated odontoblast, was inhibited by adenosine triphosphate (ATP) release channel (pannexin-1) inhibitor in a concentration- and spatial-dependent manner. Moreover, in the presence of phospholipase C (PLC) inhibitor, the increase in [Ca(2+)]i in nearby odontoblasts, following mechanical stimulation of a single odontoblast, was abolished. We could record some inward currents evoked from odontoblasts near the stimulated odontoblast, but the currents were observed in only 4.8% of the recorded odontoblasts. The results of this study showed that ATP is released via pannexin-1, from a mechanically stimulated odontoblast, which transmits a signal to nearby odontoblasts by predominant activation of PLC-coupled nucleotide receptors.

**40. Kojima Y, Higashikawa A, Kimura M, Sato M, Mochizuki H, Ogura K, Sase T, Shinya A, Kobune K, Furuya T, Sato T, Shibukawa Y, Tazaki M. Depolarization-induced Intracellular Free Calcium Concentration Increases Show No Desensitizing Effect in Rat Odontoblasts. Bull Tokyo Dent Coll. 2015;56(2):131-4.**

Odontoblasts play an important role in the transduction of the sensory signals underlying dentinal pain. Transmembrane voltage-independent Ca(2+) influx in odontoblasts has been well described. Voltage-dependent Ca(2+) influx has also been reported, but its biophysical properties remain unclear. The aim of the present study was to investigate the desensitizing effect of voltage-dependent Ca(2+) influx in rat odontoblasts by measuring depolarization-induced intracellular free Ca(2+) concentrations ([Ca(2+) ]i ). Odontoblasts on dental pulp slices from newborn rats were acutely isolated and [Ca(2+) ]i measured by using fura-2 fluorescence. Repeated application of extracellular high-K(+) solution (50 mM), which induces

membrane depolarization-elicited repeated and transient increases in [Ca(2+) ]i in the presence of extracellular Ca(2+). Increases in depolarization-induced [Ca(2+) ]i showed no significant desensitizing effect (p >0.05; Friedman test). These results suggest that odontoblasts express a voltage-dependent Ca(2+) influx pathway with no desensitizing properties.

**41. Won J, Vang H, Kim JH, Lee PR, Kang Y, Oh SB. TRPM7 Mediates Mechanosensitivity in Adult Rat Odontoblasts. J Dent Res. 2018 Feb 1:22034518759947.**

Odontoblasts, with their strategic arrangement along the outermost compartment of the dentin-pulp complex, have been suggested to have sensory function. In addition to their primary role in dentin formation, growing evidence shows that odontoblasts are capable of sensing mechanical stimulation. Previously, we found that most odontoblasts express TRPM7, the nonselective mechanosensitive ion channel reported to be critical in $Mg^{2+}$ homeostasis and dentin mineralization. In line with this finding, we sought to elucidate the functional expression of TRPM7 in odontoblasts by pharmacological approaches and mechanical stimulation. Naltriben, a TRPM7-specific agonist, induced calcium transient in the majority of odontoblasts, which was blocked by TRPM7 blockers such as extracellular $Mg^{2+}$ and FTY720 in a dose-dependent manner. Mechanical stretch of the odontoblastic membrane with hypotonic solution also induced calcium transient, which was blocked by $Gd^{3+}$, a nonselective mechanosensitive channel blocker. Calcium transient induced by hypotonic solution was also blocked by high extracellular $Mg^{2+}$ or FTY720. When TRPM7-mediated calcium transients in odontoblasts were analyzed on the subcellular level, remarkably larger transients were detected in the distal odontoblastic process compared with the soma, which was further verified with comparable immunocytochemical analysis. Our results demonstrate that TRPM7 in odontoblasts can serve as a mechanical sensor, with its distribution to facilitate intracellular $Ca^{2+}$ signaling in the odontoblastic process. These findings suggest TRPM7 as a mechanical transducer in odontoblasts to mediate intracellular calcium dynamics under diverse pathophysiological conditions of the dentin.

**42. Wen W, Que K, Zang C, Wen J, Sun G, Zhao Z, Li Y. Expression and distribution of three transient receptor potential vanilloid(TRPV) channel proteinsin human o dontoblast-like cells. J Mol Histol. 2017 Dec;48(5-6):367-377.**

Odontoblasts have been suggested to contribute to nociceptive sensation in the tooth via expression of the transient receptor potential (TRP) channels. The TRP channels as a family of nonselective cation permeable channels play an important role in sensory transduction of human. In this study, we examined the expression of transient receptor potential vanilloid-1 (TRPV1), transient receptor potential vanilloid-2 (TRPV2) and transient receptor potential vanilloid-3 (TRPV3) channels in native human odontoblasts (HODs) and long-term cultured human dental pulp cells with odontoblast phenotyoe (LHOPs) obtained from healthy wisdom teeth with the use of immunohistochemistry (IHC), immunofluorescence (IF), quantitative real-time polymerase chain reaction (qRT-PCR),western blotting (WB) and immunoelectron microscopy (IEM) assay. LHOPs samples were made into ultrathin sections, mounted on nickel grids, floated of three TRPV antibodies conjugated with 10 nm colloidal gold particles and observed under IEM at 60,000 magnifications. The relative intracellular distributions of these three channels were analyzed quantitatively on IEM images using a robust sampling, stereological estimation and statistical evaluation method. The results of IHC and IF convinced that TRPV1, TRPV2 and TRPV3 channels were expressed in native HODs and (LHOPs). The result of qRT-PCR and WB confirmed that the gene and protein expression of TRPV1, TRPV2, and TRPV3 channels and

TRPV1 mRNA are more abundantly expressed than TRPV2 and TRPV3 in HODs ($P < 0.05$). Quantitative analysis of IEM images showed that the relative intracellular distributions of these three channels are similar, and TRPV1, TRPV2 and TRPV3 proteins were preferential labeled in human odontoblast processes, mitochondria, and endoplasmic reticulum. Thus, HODs could play an important role in mediating pulp thermo-sensation due to the expression of these three TRPV channels. The difference of relative intracellular distributions of three channels suggests that special structures such as processes may have an important role to sensing of the outer stimuli first.

**43. Renard E, Gaudin A, Bienvenu G, Amiaud J, Farges JC, Cuturi MC, Moreau A, Alliot-Licht B. Immune Cells and Molecular Networks in Experimentally Induced Pulpitis. J Dent Res. 2016 Feb;95(2):196-205.**

Dental pulp is a dynamic tissue able to resist external irritation during tooth decay by using immunocompetent cells involved in innate and adaptive responses. To better understand the immune response of pulp toward gram-negative bacteria, we analyzed biological mediators and immunocompetent cells in rat incisor pulp experimentally inflamed by either lipopolysaccharide (LPS) or saline solution (phosphate-buffered saline [PBS]). Untreated teeth were used as control. Expression of pro- and anti-inflammatory cytokines, chemokine ligands, growth factors, and enzymes were evaluated at the transcript level, and the recruitment of the different leukocytes in pulp was measured by fluorescence-activated cell-sorting analysis after 3 h, 9 h, and 3 d post-PBS or post-LPS treatment. After 3 d, injured rat incisors showed pulp wound healing and production of reparative dentin in both LPS and PBS conditions, testifying to the reversible pulpitis status of this model. IL6, IL1-β, TNF-α, CCL2, CXCL1, CXCL2, MMP9, and iNOS gene expression were significantly upregulated after 3 h of LPS stimulation as compared with PBS. The immunoregulatory cytokine IL10 was also upregulated after 3 h, suggesting that LPS stimulates not only inflammation but also immunoregulation. Fluorescence-activated cell-sorting analysis revealed a significant, rapid, and transient increase in leukocyte levels 9 h after PBS and LPS stimulation. The quantity of dendritic cells was significantly upregulated with LPS versus PBS. Interestingly, we identified a myeloid-derived suppressor cell-enriched cell population in noninjured rodent incisor dental pulp. The percentage of this population, known to regulate immune response, was higher 9 h after inflammation triggered with PBS and LPS as compared with the control. Taken together, these data offer a better understanding of the mechanisms involved in the regulation of dental pulp immunity that may be elicited by gram-negative bacteria.

**44. Torres-da-Silva KR, Tessarin GWL, Dias CA, Guiati IZ, Ervolino E, Gonçalves A, Beneti IM, Lovejoy DA, Casatti CA. Teneurin-2 presence in rat and human odontoblasts. PLoS One. 2017 Sep 19;12(9):e0184794.**

Teneurins are transmembrane proteins consisting of four paralogues (Ten-1-4), notably expressed in the central nervous system during development. All teneurins contain a bioactive peptide in their carboxyl terminal named teneurin C-terminal associated peptide (TCAP). The present study analyzed the detailed distribution of teneurin-2-like immunoreactive (Ten-2-LI) cells in developing and mature rat molar teeth, as well as in mature human dental pulps. Ten-2 and TCAP-2 genic expressions were also evaluated in rat and human dental pulps. Finally, Ten-2-LI cells were analyzed during the repair process after dentin-pulp complex injury in rat lower molar teeth. For this, histological sections of rat molar teeth and human dental pulps were submitted to immunohistochemical techniques, while total RNA from developing rat teeth and mature human dental pulps were submitted to conventional RT-PCR. Ten-2-LI cells were evident in the initial bell stage of rat molar teeth development, especially in ectomesenchymal cells of the dental papilla. Ten-2-LI odontoblasts showed

strong immunoreactivity in rat and human mature teeth. Ten-2 and TCAP-2 genic expressions were confirmed in rat and human dental pulps. Dentin-pulp complex injury resulted in a decrease of Ten-2-LI odontoblasts after traumatic injury. Interestingly, Ten-2-LI cells were also evident in the pulp cell-rich zone in all postoperative days. In conclusion, Ten-2-LI presence in rat and human odontoblasts was demonstrated for the first time and Ten-2/TCAP-2 genic expressions were confirmed in rat and human dental pulps. Furthermore, it was revealed that Ten-2-LI rat odontoblasts can be modulated during the regenerative process.

**45. Kim YS[1], Jung HK, Kwon TK, Kim CS, Cho JH, Ahn DK, Bae YC. Expression of transient receptor potential ankyrin 1 in human dental pulp. J Endod. 2012 Aug;38(8):1087-92.**

Introduction: Transient receptor potential ankyrin 1 (TRPA1) is activated by noxious cold (<17°C) and contributes to cold and mechanical hypersensitivity after inflammation and nerve injury. Methods: To investigate whether TRPA1 is involved in the mediation of nociception, including noxious cold and cold hypersensitivity in teeth, we examined the expression of TRPA1 and sodium channel Nav1.8 in human dental pulp using fluorescent and electron microscopic immunocytochemistry. Results: TRPA1 was expressed in a large number of axons branching extensively in the peripheral pulp and in a few axons within the nerve bundles in the core of the coronal pulp and in the radicular pulp. Under electron microscopy, TRPA1 immunoreactivity was typically localized near the plasma membrane of unmyelinated axons in the peripheral pulp, suggesting that in these axons it may act as a functional receptor. The proportion of axons expressing TRPA1 in neurofilament 200-positive axons significantly increased in the painful pulp compared with the normal pulp. TRPA1 was also densely expressed in the processes and the cell body of odontoblasts. A large number of axons coexpressed TRPA1 and Nav1.8. Conclusions: These findings support the notion that TRPA1 is involved in the perception of noxious cold and cold hypersensitivity in human dental pulp and that TRPA1-mediated nociception is primarily mediated by axons and odontoblasts in the peripheral pulp.

**Tabla 4. Preselección de artículos por temática**

**SCIENCE DIRECT**

| | |
|---|---|
| **TEMATICA** | **LA OTRA VISIÓN DEL ODONTOBLASTO** |
| **BASE DE DATOS** | **SCIENCE DIRECT** |
| **ALGORITMO FINAL** | Odontoblasts, Odontoblasts AND nociception, Odontoblasts AND immunology OR immunology AND inflammation. |

**artículos preseleccionados**

Referencia -estilo Vancouver y abstract

**1. El Karim IA, McCrudden MT, McGahon MK, Curtis TM, Jeanneau C, Giraud T, Irwin CR, Linden GJ, Lundy FT, About I. Biodentine Reduces Tumor Necrosis Factor Alpha-induced TRPA1 Expression in OdontoblastlikeCells. J Endod. 2016 Apr; 42(4):589-95.**

Introduction: The transient receptor potential (TRP) ion channels have emerged as important cellular sensors in both neuronal and non-neuronal cells, with TRPA1 playing a central role in nociception and neurogenic inflammation. The

functionality of TRP channels has been shown to be modulated by inflammatory cytokines. The aim of this study was to investigate the effect of inflammation on odontoblast TRPA1expression and to determine the effect of Biodentine (Septodent, Paris, France) on inflammatory-induced TRPA1 expression. Methods: Immunohistochemistry was used to study TRPA1 expression in pulp tissue from healthy and carious human teeth. Pulp cellswere differentiated to odontoblastlike cells in the presence of 2 mmol/L beta-glycerophosphate, and these cells were used in quantitative polymerase chain reaction, Western blotting, calcium imaging, and patch clamp studies. Results: Immunofluorescent staining revealed TRPA1 expression in odontoblast cell bodies and odontoblast processes, which was more intense in carious versus healthy teeth. TRPA1 gene expression was induced in cultured odontoblastlike cells by tumor necrosis factor alpha, and this expression was significantly reduced in the presence of Biodentine. The functionality of the TRPA1 channel was shown by calcium microfluorimetry and patch clamp recording, and our results showed a significant reduction in tumor necrosis factor alpha-induced TRPA1responses after Biodentine treatment. Conclusions: In conclusion, this study showed TRPA1 to be modulated by caries-induced inflammation and that Biodentine reduced TRPA1 expression and functional responses.

**2. Cooper PR, Chicca IJ, Holder MJ, Milward MR. Inflammation and Regeneration in the Dentin-pulp Complex: Net Gain or Net Loss?. J Endod. 2017 Sep; 43(9S):S87-S94.**

The balance between the immune/inflammatory and regenerative responses in the diseased pulp is central to the clinical outcome, and this response is unique within the body because of its tissue site. Cariogenic bacteria invade the dentin and pulp tissues, triggering molecular and cellular events dependent on the disease stage. At the early onset, odontoblasts respond to bacterial components in an attempt to protect the tooth's hard and soft tissues and limit disease progression. However, as disease advances, the odontoblasts die, and cells central to the pulp core, including resident immune cells, pulpal fibroblasts, endothelial cells, and stem cells, respond to the bacterial challenge via their expression of a range of pattern recognition receptors that identify pathogen-associated molecular patterns. Subsequently, recruitment and activation occurs of a range of immune cell types, including neutrophils, macrophages, and T and B cells, which are attracted to the diseased site by cytokine/chemokine chemotactic gradients initially generated by resident pulpal cells. Although these cells aim to disinfect the tooth, their extravasation, migration, and antibacterial activity (eg, release of reactive oxygen species [ROS]) along with the bacterial toxins cause pulp damage and impede tissue regeneration processes. Recently, a novel bacterial killing mechanism termed neutrophil extracellular traps (NETs) has also been described that uses ROS signaling and results in cellular DNA extrusion. The NETs are decorated with antimicrobial peptides (AMPs), and their interaction with bacteria results in microbial entrapment and death. Recent data show that NETs can be stimulated by bacteria associated with endodontic infections, and they may be present in inflamed pulp tissue. Interestingly, some bacteria associated with pulpal infections express deoxyribonuclease enzymes, which may enable their evasion of NETs. Furthermore, although NETs aim to localize and kill invading bacteria using AMPs and histones, limiting the spread of the infection, data also indicate that NETs can exacerbate inflammation and their components are cytotoxic. This review considers the potential role of NETs within pulpal infections and how these structures may influence the pulp's vitality and regenerative responses.

**3. Cooper PR, Holder MJ, Smith AJ. Inflammation and regeneration in the dentin-pulp complex: a double-edged sword. J Endod. 2014 Apr;40(4 Suppl):S46-51.**

Dental tissue infection and disease result in acute and chronic activation of the innate immune response, which is mediated by molecular and cellular signaling. Different cell types within the dentin-pulp complex are able to detect invading bacteria at all stages of the infection. Indeed, at relatively early disease stages, odontoblasts will respond to bacterial components, and as the disease progresses, core pulpal cells including fibroblasts, stems cells, endothelial cells, and immune cells will become involved. Pattern recognition receptors, such as Toll-like receptors expressed on these cell types, are responsible for detecting bacterial components, and their ligand binding leads to the activation of the nuclear factor-kappa B and p38 mitogen-activated protein (MAP) kinase intracellular signaling cascades. Subsequent nuclear translocation of the transcription factor subunits from these pathways will lead to proinflammatory mediator expression, including increases in cytokines and chemokines, which trigger host cellular defense mechanisms. The complex molecular signaling will result in the recruitment of immune system cells targeted at combating the invading microbes; however, the trafficking and antibacterial activity of these cells can lead to collateral tissue damage. Recent evidence suggests that if inflammation is resolved relatively low levels of proinflammatory mediators may promote tissue repair, whereas if chronic inflammation ensues repair mechanisms become inhibited. Thus, the effects of mediators are temporal context dependent. Although containment and removal of the infection are keys to enable dental tissue repair, it is feasible that the development of anti-inflammatory and immunomodulatory approaches, based on molecular, epigenetic, and photobiomodulatory technologies, may also be beneficial for future endodontic treatments.

**4. Nishiyama A, Sato M, Kimura M, Intercellular signal communication among. Cell Calcium. 2016 Nov; 60 (5): 341-355.**

Various stimuli to the exposed surface of dentin induce changes in the hydrodynamic force inside the dentinal tubules resulting in dentinal pain. Recent evidences indicate that mechano-sensor channels, such as the transient receptor potential channels, in odontoblasts receive these hydrodynamic forces and trigger the release of ATP to the pulpal neurons, to generate dentinal pain. A recent study, however, has shown that odontoblasts also express glutamate receptors (GluRs). This implies that cells in the dental pulp tissue have the ability to release glutamate, which acts as a functional intercellular mediator to establish inter-odontoblast and odontoblast-trigeminal ganglion (TG) neuron signal communication. To investigate the intercellular signal communication, we applied mechanical stimulation to odontoblasts and measured the intracellular free $Ca^{2+}$concentration ($[Ca^{2+}]_i$). During mechanical stimulation in the presence of extracellular $Ca^{2+}$, we observed a transient $[Ca^{2+}]_i$ increase not only in single stimulated odontoblasts, but also in adjacent odontoblasts. We could not observe these responses in the absence of extracellular $Ca^{2+}$. $[Ca^{2+}]_i$ increases in the neighboring odontoblasts during mechanical stimulation of single odontoblasts were inhibited by antagonists of metabotropic glutamate receptors (mGluRs) as well as glutamate-permeable anion channels. In the odontoblast-TG neuron coculture, we observed an increase in $[Ca^{2+}]_i$ in the stimulated odontoblasts and TG neurons, in response to direct mechanical stimulation of single odontoblasts. These $[Ca^{2+}]_i$increases in the neighboring TG neurons were inhibited by antagonists for mGluRs. The $[Ca^{2+}]_i$ increases in the stimulated odontoblasts were also inhibited by mGluRs antagonists. We further confirmed that the odontoblasts express group I, II, and III mGluRs. However, we could not record any currents evoked from odontoblasts near the mechanically stimulated odontoblast, with or without extracellular $Mg^{2+}$, indicating that N-methyl-d-aspartic acid receptor does not contribute to inter-odontoblast signal communication. The results suggest that a mechanically stimulated odontoblast is capable of releasing glutamate into the extracellular space via glutamate-permeable anion channels. The released glutamate activates mGluRs on the odontoblasts in an autocrine/paracrine manner, forming an inter-odontoblasts communication, which drives dentin formation via odontoblast-odontoblast signal communication. Glutamate and mGluRs

also mediate neurotransmission between the odontoblasts and neurons in the dental pulp to modulate sensory signal transmission for dentinal sensitivity.

**5. Cho YS, Ryu CH, Won JH, Vang H, Oh SB, Ro JY, Bae YC. Rat odontoblasts may use glutamate to signal dentin injury. Neuroscience. 2016 Oct 29;335:54-63.**

Accumulating evidence indicates that odontoblasts act as sensor cells, capable of triggering action potentials in adjacent pulpal nociceptive axons, suggesting a paracrine signaling via a currently unknown mediator. Since glutamate can mediate signaling by non-neuronal cells, and peripheral axons may express glutamate receptors (GluR), we hypothesized that the expression of high levels of glutamate, and of sensory receptors in odontoblasts, combined with an expression of GluR in adjacent pulpal axons, is the morphological basis for odontoblastic sensory signaling. To test this hypothesis, we investigated the expression of glutamate, the thermo- and mechanosensitive ion channels transient receptor potential vanilloid 1 (TRPV1), transient receptor potential ankyrin 1 (TRPA1), and TWIK-1-related K+channel (TREK-1), and the glutamate receptor mGluR5, in a normal rat dental pulp, and following dentin injury. We also examined the glutamate release from odontoblast in cell culture. Odontoblasts were enriched with glutamate, at the level as high as in adjacent pulpal axons, and showed immunoreactivity for TRPV1, TRPA1, and TREK-1. Pulpal sensory axons adjacent to odontoblasts expressed mGluR5. Both the levels of glutamate in odontoblasts, and the expression of mGluR5 in nearby axons, were upregulated following dentin injury. The extracellular glutamate concentration was increased significantly after treating of odontoblast cell line with calcium permeable ionophore, suggesting glutamate release from odontoblasts. These findings lend morphological support to the hypothesis that odontoblasts contain glutamate as a potential neuroactive substance that may activate adjacent pulpal axons, and thus contribute to dental pain and hypersensitivity.

**6. Ikeda H, Suda H. Odontoblastic syncytium through electrical coupling in the human dental pulp. J Dent Res. 2013 Apr;92(4):371-5.**

We have previously reported a dye-coupling network between odontoblasts (OBs). However, it is still unclear how the information detected by the odontoblasts is transmitted. The aim of this study was to characterize the odontoblastic syncytium electrophysiologically in the humandental pulp. Pulpal cells were freshly isolated from human premolars immediately after extraction. Under a light microscope, coupled or small clusters (3-20) of odontoblasts, each of which had a monopolar process (95-280 μm) and an oval cell body, were easily observed to be lined up in parallel. Cells were used for electrophysiological recording within 3 hrs in the dual patch-clamp configuration. Electrical couplings were found between odontoblasts (37/40 pairs). Voltage gating showed directional independence between pairs of odontoblasts. The time constant to a current decay increased with the number of clustered odontoblasts. Nine of 37 pairs isolated from young patients were electrically coupled, but could not be voltage-clamped. Transjunctional currents were blocked by octanol. These results suggest that odontoblasts form a syncytium that is directionally independent via symmetric gap junction channels in the odontoblastic layer. Young odontoblasts with a high electrical conductance to neighboring cells may be related to high potential of information transmission or calcification.

7. **El Karin IA, Linden GJ, Curtis TM, About I, McGahon MK, Irwin CR, Lundy FT. Human odontoblasts express functional thermo-sensitive TRP channels: implications for dentin sensitivity. Pain. 2011 Oct;152(10):2211-23.**

Odontoblasts form the outermost cellular layer of the dental pulp where they have been proposed to act as sensory receptor cells. Despite this suggestion, evidence supporting their direct role in mediating thermo-sensation and nociception is lacking. Transient receptor potential (TRP) ion channels directly mediate nociceptive functions, but their functional expression in human odontoblasts has yet to be elucidated. In the present study, we have examined the molecular and functional expression of thermo-sensitive TRP channels in cultured odontoblast-like cells and in native human odontoblasts obtained from healthy wisdom teeth. PCR and western blotting confirmed gene and protein expression of TRPV1, TRPA1 and TRPM8 channels. Immunohistochemistry revealed that these channels were localised to odontoblast-like cells as determined by double staining with dentin sialoprotein (DSP) antibody. In functional assays, agonists of TRPV1, TRPA1 and TRPM8 channels elicited [Ca2+]i transients that could be blocked by relevant antagonists. Application of hot and cold stimuli to the cells also evoked rises in [Ca2+]i which could be blocked by TRP-channel antagonists. Using a gene silencing approached we further confirmed a role for TRPA1 in mediating noxious cold responses in odontoblasts. We conclude that human odontoblasts express functional TRP channels that may play a crucial role in mediating thermal sensation in teeth. Cultured and native human odontoblasts express functional TRP channels that may play a crucial role in mediating thermal sensation in teeth.

8. **Solé-Magdalena A, Martínez-Alonso M, Coronado CA, Junquera LM, Cobo J, Vega JA. Molecular basis of dental sensitivity: The odontoblasts are multisensory cells and express multifunctional ion channels. Ann Anat. 2018 Jan;215:20-29.**

Odontoblasts are the dental pulp cells responsible for the formation of dentin. In addition, accumulating data strongly suggest that they can also function as sensory cells that mediate the early steps of mechanical, thermic, and chemical dental sensitivity. This assumption is based on the expression of different families of ion channels involved in various modalities of sensitivity and the release of putative neurotransmitters in response to odontoblast stimulation which are able to act on pulp sensory nerve fibers. This review updates the current knowledge on the expression of transient-potential receptor ion channels and acid-sensing ion channels in odontoblasts, nerve fibers innervating them and trigeminal sensory neurons, as well as in pulp cells. Moreover, the innervation of the odontoblasts and the interrelationship been odontoblasts and nerve fibers mediated by neurotransmitters was also revisited. These data might provide the basis for novel therapeutic approaches for the treatment of dentin sensibility and/or dental pain.

9. **Tokuda M, Tatsuyama S, Fujisawa M, Morimoto-Yamashita Y, Kawakami Y, Shibukawa Y, Torii M. Dentin and pulp sense cold stimulus. Med Hypotheses. 2015 May;84(5):442-4.**

Dentin hypersensitivity is a common symptom, and recent convergent evidences have reported transient receptor potential (TRP) channels in odontoblasts act as mechanical and thermal molecular sensor, which detect stimulation applied on the exposed dentin surface, to drive multiple odontoblastic cellular functions, such as sensory transduction and/or dentin formation. In the present study, we confirmed expression of TRP melastatin subfamily member-8 (TRPM8)

channels in primary cultured cells derived from human dental pulp cells (HPCs) and mouse odontoblast-lineage cells (OLCs) as well as in dentin matrix protein-1 (DMP-1) and dentin sialoprotein (DSP) positive acutely isolated rat odontoblasts from dental pulp tissue slice culture by immunohistochemical analyses. In addition, we detected TRPM8 channel expression on HPCs and OLCs by RT-PCR and Western blotting analyses. These results indicated that both odontoblasts and dental pulp cells express TRPM8 channels in rat, mouse and human, and therefore we hypothesize they may contribute as cold sensor in tooth.

10. **Song Z, Chen L, Guo J, Qin W, Wang R, Huang S, Yang X, Tian Y, Lin Z. The Role of Transient Receptor Potential Cation Channel, Subfamily C, Member 1 in the Odontoblast-like Differentiation of Human Dental Pulp Cells. J Endod. 2017 Feb;43(2):315-320.**

Introduction: Calcium ions ($Ca^{2+}$) actively participate in reparative dentin formation by promoting cellular proliferation and differentiationof human dental pulp cells (hDPCs). Transient receptor. Methods: Immunohistochemical staining was used to determine the distribution of TRPC1 in pulp tissues. Western blot analysis was used to detect the protein level of TRPC1 in the odontoblast-like differentiation of hDPCs. Knockdown of TRPC1 was performed with an adenoviral vector to evaluate the role of TRPC1 in hDPCs during odontoblast-like differentiation. Results: The results showed that TRPC1 was highly expressed in the cytoplasm of dental pulp cells, especially in the odontoblast layer of the healthy pulp. Moreover, the protein level of TRPC1 increased in a time-dependent manner during the odontoblast-like differentiation of hDPCs. Importantly, knockdown of TRPC1 attenuated the process of odontoblast-like differentiation as indicated by the reduction in mineralized nodules and the down-regulation of dentin sialophosphoprotein and dentin matrix protein 1. Moreover, knockdown of TRPC1 decreased $Ca^{2+}$ entry to the cytoplasm of hDPCs. Conclusions: Our data indicated a pivotal role of TRPC1 in the odontoblastlike differentiation of hDPCs, which may be a therapeutic target to enhance reparative dentin formation.

11. **Le Fur-Bonnabesse A, Bodéré C, Hélou C, Chevalier V, Goulet JP. Dental pain induced by an ambient thermal differential: pathophysiological hypothesis. J Pain Res. 2017 Dec 15;10:2845-2851.**

Dental pain triggered by temperature differential is a misrecognized condition and a form of dental allodynia. Dental allodynia is characterized by recurrent episodes of diffuse, dull and throbbing tooth pain that develops when returning to an indoor room temperature after being exposed for a long period to cold weather. The pain episode may last up to few hours before subsiding. Effective treatment is to properly shield the pulpal tissue of the offending tooth by increasing the protective layer of the dentin/enamel complex. This review underscores the difference in dentin hypersensitivity and offers a mechanistic hypothesis based on the following processes. Repeated exposure to significant positive temperature gradients (from cold to warm) generates phenotypic changes of dental primary afferents on selected teeth with subsequent development of a "low-grade" neurogenic inflammation. As a result, nociceptive C-fibers become sensitized and responsive to innocuous temperature gradients because the activation threshold of specific TRP ion channels is lowered and central sensitization takes place. Comprehensive overviews that cover dental innervation and sensory modalities, thermodynamics of tooth structure, mechanisms of dental nociception and the thermal pain are also provided.

12. **Kim HJ, Shuprisha A, Shikano T, Tsumura M, Shibukawa Y, Tazaki M. Voltage-dependent sodium channels and calcium-activated potassium channels in humanodontoblasts in vitro. J Endod. 2012 Oct;38(10):1355-62.**

Introduction: Transmembrane ionic signaling regulates many cellular processes in both physiological and pathologic settings. In this study, the biophysical properties of voltage-dependent Na(+) channels in odontoblasts derived from human dental pulp (HOB cells) were investigated together with the effect of bradykinin on intracellular Ca(2+) signaling and expression of Ca(2+)-activated K(+) channels. Methods: Ionic channel activity was characterized by using whole-cell patch-clamp recording and fura-2 fluorescence. Results: Mean resting membrane potential in the HOB cells was -38 mv. Depolarizing steps from a holding potential of -80 mv activated transient voltage-dependent inward currents with rapid activation/inactivation properties. At a holding potential of -50 mv, no inward current was recorded. Fast-activation kinetics exhibited dependence on membrane potential, whereas fast-inactivation kinetics did not. Steady-state inactivation was described by a Boltzmann function with a half-maximal inactivation potential of -70 mv, indicating that whereas the channels were completely inactivated at physiological resting membrane potential, they could be activated when the cells were hyperpolarized. Inward currents disappeared in Na(+)-free extracellular solution. Bradykinin activated intracellular Ca(2+)-releasing and influx pathways. When the HOB cells were clamped at a holding potential of -50 mv, outward currents were recorded at positive potentials, indicating sensitivity to inhibitors of intermediate-conductance Ca(2+)-activated K(+) channels. Conclusions: Human odontoblasts expressed voltage-dependent Na(+) channels, bradykinin receptors, and Ca(2+)-activated K(+) channels, which play an important role in driving cellular functions by channel-receptor signal interaction and membrane potential regulation.

13. **Tsumura M, Sobhan U, Muramatsu T, Sato M, Ichikawa H, Sahara Y, Tazaki M, Shibukawa Y. TRPV1-mediated calcium signal couples with cannabinoid receptors and sodium calcium exchangers in rat odontoblasts. Cell Calcium. 2012 Aug;52(2):124-36.**

Odontoblasts are involved in the transduction of stimuli applied to exposed dentin. Although expression of thermo/mechano/osmo-sensitive transient receptor potential (TRP) channels has been demonstrated, the properties of TRP vanilloid 1 (TRPV1)-mediated signaling remain to be clarified. We investigated physiological and pharmacological properties of TRPV1 and its functional coupling with cannabinoid (CB) receptors and Na(+)-Ca(2+) exchangers (NCXs) in odontoblasts. Anandamide (AEA), capsaicin (CAP), resiniferatoxin (RF) or low-pH evoked Ca(2+) influx. This influx was inhibited by capsazepine (CPZ). Delay in time-to-activation of TRPV1 channels was observed between application of AEA or CAP and increase in [Ca(2+)](i). In the absence of extracellular Ca(2+), however, an immediate increase in [Ca(2+)](i) was observed on administration of extracellular Ca(2+), followed by activation of TRPV1 channels. Intracellular application of CAP elicited inward current via opening of TRPV1 channels faster than extracellular application. With extracellular RF application, no time delay was observed in either increase in [Ca(2+)](i) or inward current, indicating that agonist binding sites are located on both extra- and intracellular domains. KB-R7943, an NCX inhibitor, yielded an increase in the decay time constant during TRPV1-mediated Ca(2+) entry. Increase in [Ca(2+)](i) by CB receptor agonist, 2-arachidonylglycerol, was inhibited by CB1 receptor antagonist or CPZ, as well as by adenylyl cyclase inhibitor. These results showed that TRPV1-mediated Ca(2+) entry functionally couples with CB1 receptor activation via cAMP signaling. Increased [Ca(2+)](i) by TRPV1 activation was extruded by NCXs. Taken together, this suggests that cAMP-mediated CB1-TRPV1

crosstalk and TRPV1-NCX coupling play an important role in driving cellular functions following transduction of external stimuli to odontoblasts.

## ARTÍCULOS RELACIONADOS ENCONTRADOS

Listado de artículos Referencia -estilo Vancouver y abstract

1. **Tazawa K, Ikeda H, Kawashima N, Okiji T. Transient receptor potential melastatin (TRPM) 8 is expressed in freshly isolated native humanodontoblasts . Arch Oral Biol. 2017 Mar;75:55-61.**

Objective: Cold-sensitive ion channels, such as transient receptor potential melastatin (TRPM) 8 and transient receptor potential ankyrin (TRPA) 1, may play a crucial role in the nociceptive function of odontoblasts, whereas expression of these TRP channels in human native odontoblasts remains to be elucidated. This study aimed to analyze the expression of TRPM8 and TRPA1 in freshly isolated native human odontoblasts. Design: Odontoblasts were isolated from freshly extracted healthy human teeth (n=4); after removing the inner pulp tissues from the pulp chambers, odontoblasts remaining on the dentin surface were washed out with phosphate buffered saline and collected. Reverse transcription-polymerase chain reaction was employed to compare the expression levels of TRPM8, TRPA1, and dentin matrix acidic phosphoprotein 1 (DMP1) mRNAs between the isolated odontoblasts and the inner pulp tissues. The isolated cells were subjected to immunolocalization of TRPM8 and nestin. Paraformaldehyde-fixed, EDTA-demineralized frozen sections obtained from freshly extracted healthy human teeth (n=4) were also analyzed immunohistochemically using anti-nestin, TRPM8, and TRPA1 antibodies. Results: Expression levels of TRPM8 and DMP1 in the isolated odontoblasts were significantly higher than those in the inner pulp tissues ($p<0.05$). Expression of TRPM8 and nestin was observed in the odontoblastic layer of the dental pulp tissue and isolated odontoblasts, while expression of TRPA1 was not detected. Conclusions: TRPM8, but not TRPA1, was detected in freshly isolated native human odontoblasts at the protein and mRNA levels, suggesting that odontoblasts play an important role in detecting external cold stimulation via TRPM8 in healthy condition.

2. **Kwon M, Baek SH, Park CK, Chung G, Oh SB. Single-cell RT-PCR and immunocytochemical detection of mechanosensitive transient receptorpotential channels in acutel y isolated rat odontoblasts. Arch Oral Biol. 2014 Dec;59(12):1266-71.**

Objective: Hydrostatic force applied to tooth pulp has long been suspected to be the direct cause of dental pain. However, the molecular and cellular identity of the transducer of the mechanical force in teeth is not clear. Growing number of literatures suggested that odontoblasts, secondary to its primary role as formation of tooth structure, might function as a cellular mechanical transducer in teeth. Design: In order to determine whether odontoblasts could play a crucial role in transduction of hydrostatic force applied to dental pulp into electrical impulses, current study investigated the expression of stretch-activated transient receptor potential (TRP) channels in acutelyisolated odontoblasts from adult rats by single cell reverse transcriptase polymerase chain reaction and immunocytochemical analysis. Results: As the result, expression of TRPM7 (melastatin 7) was observed in majority (87%) of odontoblasts while mRNAs for TRPC1 (canonical 1), TRPC6 (canonical 6) and TRPV4 (vanilloid 4) were detected in small subpopulations of odontoblasts. TRPM3 (melastatin 3) was not detected in our experimental set-up. Immunocytochemical analysis further revealed TRPM7 expression at protein level. Conclusion: Expression of the mechanosensitive TRP channels provides additional evidence that supports the

sensory roles of odontoblasts. Given that TRPM7 is a mechanosensitive ion channel with a kinase activity that plays a role in Mg(2+) homeostasis, it is possible that TRPM7 expressed in odontoblasts might play a central role in mineralization during dentin formation.

**3. Chung G, Jung SJ, Oh SB. Cellular and molecular mechanisms of dental nociception. J Dent Res. 2013 Nov;92(11):948-55.**

Due, in part, to the unique structure of the tooth, dental pain is initiated via distinct mechanisms. Here we review recent advances in our understanding of inflammatory tooth pain and discuss 3 hypotheses proposed to explain dentinal hypersensitivity: The first hypothesis, supported by functional expression of temperature-sensitive transient receptor potential channels, emphasizes the direct transduction of noxious temperatures by dental primary afferent neurons. The second hypothesis, known as hydrodynamic theory, attributes dental pain to fluid movement within dentinal tubules, and we discuss several candidate cellular mechanical transducers for the detection of fluid movement. The third hypothesis focuses on the potential sensory function of odontoblasts in the detection of thermal or mechanical stimuli, and we discuss the accumulating evidence that supports their excitability. We also briefly update on a novel strategy for local nociceptive anesthesia via nociceptive transducer molecules in dental primary afferents with the potential to specifically silence pain fibers during dental treatment. Further understanding of the molecular mechanisms of dental pain would greatly enhance the development of therapeutics that target dental pain.

**4. Khatibi Shahidi M, Krivanek J, Kaukua N, Ernfors P, Hladik L, Kostal V, Masich S, Hampl A, Chubanov V, Gudermann T, Romanov RA, Harkany T, Adameyko I, Fried K. Three-dimensional Imaging Reveals New Compartments and Structural Adaptations in Odontoblasts. J Dent Res. 2015 Jul;94(7):945-54.**

In organized tissues, the precise geometry and the overall shape are critical for the specialized functions that the cells carry out. Odontoblasts are major matrix-producing cells of the tooth and have also been suggested to participate in sensory transmission. However, refined morphologic data on these important cells are limited, which hampers the analysis and understanding of their cellular functions. We took advantage of fluorescent color-coding genetic tracing to visualize and reconstruct in 3 dimensions single odontoblasts, pulp cells, and their assemblages. Our results show distinct structural features and compartments of odontoblasts at different stages of maturation, with regard to overall cellular shape, formation of the main process, orientation, and matrix deposition. We demonstrate previously unanticipated contacts between the processes of pulp cells and odontoblasts. All reported data are related to mouse incisor tooth. We also show that odontoblasts express TRPM5 and Piezo2 ion channels. Piezo2 is expressed ubiquitously, while TRPM5 is asymmetrically distributed with distinct localization to regions proximal to and within odontoblast processes.

**5. Que K, He D, Jin Y, Wu L, Wang F, Zhao Z, Yang J, Deng J. Expression of Cannabinoid Type 1 Receptors in Human Odontoblast Cells. J Endod. 2017 Feb;43(2):283-288.**

Introduction: The aim of this study was to investigate the functional expression of cannabinoid type 1 (CB1) receptors in humanodontoblasts (HODs) and the possible internal mechanism. Methods: In the present study, we examined the molecular and functional expression of the CB1 receptors in

cultured HOD-like cells and native HODs obtained from healthy wisdom teeth. Results: Immunohistochemistry and immunofluorescence revealed that CB1 receptors localize to native HODs and HOD-like cells, respectively. Both reverse-transcription polymerase chain reaction and Western blot analysis confirmed gene and protein expression of CB1 receptors. The ultrastructural distribution by immunoelectron microscopy also found that CB1 receptors labeled by colloidal gold particles distribute sparsely in the cytoplasm and odontoblastic processes. In functional assays, 2-arachidonyl glycerol, as an agonist of CB receptors, elicited the increase of intracellular fluorescence intensity that could be inhibited by a CB1-specific receptor antagonist rather than a selective CB2 receptor antagonist with fluo-3AM $Ca^{2+}$ fluorescence. The source of the increase of intracellular fluorescence intensity elicited by CB1 receptors was from extracellular $Ca^{2+}$ but not intracellular $Ca^{2+}$ stores. The process of 2-arachidonyl glycerol activating CB1 receptors modulated transient receptor potential vanilloid 1-mediated $Ca^{2+}$ entry via the cyclic adenosine monophosphate signaling pathway. Conclusions: We conclude that HODs can express functional CB1 receptors that may play an important role in mediating the physiological function in tooth pulp.

**6. Egbuniwe O, Grover S, Duggal AK, Mavroudis A, Yazdi M, Renton T, Di Silvio L, Grant AD. TRPA1 and TRPV4 activation in human odontoblasts stimulates ATP release. J Dent Res. 2014 Sep;93(9):911**

The mechanism of pain in dentine hypersensitivity is poorly understood but proposed to result from the activation of dental sensory neurons in response to dentinal fluid movements. Odontoblasts have been suggested to contribute to thermal and mechanosensation in the tooth via expression of transient receptor potential (TRP) channels. However, a mechanism by which odontoblasts could modulate neuronal activity has not been demonstrated. In this study, we investigated functional TRP channel expression in human odontoblast-like cells and measured ATP release in response to TRP channel activation. Human immortalized dental pulp cells were driven toward an odontoblast phenotype by culture in conditioned media. Functional expression of TRP channels was determined with reverse transcription polymerase chain reaction and ratiometric calcium imaging with Fura-2. ATP release was measured using a luciferin-luciferase assay. Expression of mRNA for TRPA1, TRPV1, and TRPV4 but not TRPM8 was detected in odontoblasts by reverse transcription polymerase chain reaction. Expression of TRPV4protein was detected by Western blotting and immunocytochemistry. The TRPA1 agonists allyl isothiocyanate and cinnamaldehyde and the TRPV4 agonist GSK1016790A caused a concentration-dependent increase in intracellular Ca(2+) concentration that was inhibited by the selective antagonists HC030031, AP18, and HC067047, respectively. In contrast, exposure to the TRPV1 agonist capsaicin or the TRPM8 agonist icilin had no effect on intracellular Ca(2+) concentration. Treatment with allyl isothiocyanate, cinnamaldehyde, or GSK1016790A caused an increase in ATP concentration in culture medium that was abolished by preincubation with TRP channel antagonists. These data demonstrate that activation of TRPA1 and TRPV4 channels in human odontoblast-like cells can stimulate ATP release. We were unable to confirm the presence of thermosensitive TRPV1 and TRPM8 that has previously been reported in odontoblasts.

**7. Fu D, Song F, Sun H, Pei D, Wang Y, Lei J, Huang C. Expression of Pannexin3 in human odontoblast-like cells and its hemichannel function in mediating ATP release. Arch Oral Biol. 2015 Oct;60(10):1510-6.**

Objective: The aim of this study is to investigate the expression of pannexin3 (Panx3) in human odontoblast-like cells (hOBs) and its hemichannel function in mediating ATP release. Methods: RT-PCR and immunofluorescence analysis were used to detect the expression of pannexins (Panxs) in human dental pulp tissue and cultured cells. To

determine the role of Panx3 in ATP release, hOBs were infected with Panx3-overexpression lentivirus, Panx3-shRNA lentivirus or control lentivirus and then stimulated with cold buffer. Intracellular ATP was monitored using quinacrine, and then semi-quantitatively analyzed. In the meantime, the ATP release was quantitatively analyzed using the bioluminescence method when the cells were exposed to cold stimulus. Results: Panx3 mRNA and protein were found in dental pulp tissue and cultured cells. Upon cold stimulus, intracellular ATP was released into the extracellular space. Overexpression of Panx3 accelerated ATP release, whereas inhibition of Panx3 suppressed this process. Conclusion: Panx3 hemichannel is expressed in human odontoblast-like cells and mediates ATP release into the extracellular space.

**8. Liu X, Wang C, Fujita T, Malmstrom HS, Nedergaard M, Ren YF, Dirksen RT. External Dentin Stimulation Induces ATP Release in Human Teeth. J Dent Res. 2015 Sep;94(9):1259-66.**

ATP is involved in neurosensory processing, including nociceptive transduction. Thus, ATP signaling may participate in dentin hypersensitivity and dental pain. In this study, we investigated whether pannexins, which can form mechanosensitive ATP-permeable channels, are present in human dental pulp. We also assessed the existence and functional activity of ecto-ATPase for extracellular ATP degradation. We further tested if ATP is released from dental pulp upon dentin mechanical or thermal stimulation that induces dentin hypersensitivity and dental pain and if pannexin or pannexin/gap junction channel blockers reduce stimulation-dependent ATP release. Using immunofluorescence staining, we demonstrated immunoreactivity of pannexin 1 and 2 in odontoblasts and their processes extending into the dentin tubules. Using enzymatic histochemistry staining, we also demonstrated functional ecto-ATPase activity within the odontoblast layer, sub odontoblast layer, dental pulp nerve bundles, and blood vessels. Using an ATP bioluminescence assay, we found that mechanical or cold stimulation to the exposed dentin induced ATP release in an in vitro human tooth perfusion model. We further demonstrated that blocking pannexin/gap junction channels with probenecid or carbenoxolone significantly reduced external dentin stimulation-induced ATP release. Our results provide evidence for the existence of functional machinery required for ATP release and degradation in human dental pulp and that pannexin channels are involved in external dentin stimulation-induced ATP release. These findings support a plausible role for ATP signaling in dentin hypersensitivity and dental pain.

**9. Lee BM, Jo H, Park G, Kim YH, Park CK, Jung SJ, Chung G, Oh SB. Extracellular ATP Induces Calcium Signaling in Odontoblasts. J Dent Res. 2017 Feb;96(2):200-207.**

Odontoblasts form dentin at the outermost surface of tooth pulp. An increasing level of evidence in recent years, along with their locational advantage, implicates odontoblasts as a secondary role as sensory or immune cells. Extracellular adenosine triphosphate (ATP) is a well-characterized signaling molecule in the neuronal and immune systems, and its potential involvement in inter odontoblast communications was recently demonstrated. In an effort to elaborate the ATP-mediated signaling pathway in odontoblasts, the current study performed single-cell reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescent detection to investigate the expression of ATP receptors related to calcium signal in odontoblasts from incisal teeth of 8- to 10-wk-old rats, and demonstrated an in vitro response to ATP application via calcium imaging experiments. While whole tissue RT-PCR analysis detected $P2Y_2$, $P2Y_4$, and all 7 subtypes ($P2X_1$ to $P2X_7$) in tooth pulp, single-cell RT-PCR analysis of acutely isolated rat odontoblasts revealed $P2Y_2$, $P2Y_4$, $P2X_2$, $P2X_4$, $P2X_6$, and $P2X_7$ expression in only a subset (23% to 47%) of cells tested, with no evidence for $P2X_1$, $P2X_3$, and $P2X_5$ expression. An increase of intracellular $Ca^{2+}$ concentration in response

to 100μM ATP, which was repeated after pretreatment of thapsigargin or under the $Ca^{2+}$-free condition, suggested function of both ionotropic and metabotropic ATP receptors in odontoblasts. The enhancement of ATP-induced calcium response by ivermectin and inhibition by 5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD) confirmed a functional $P2X_4$ subtype in odontoblasts. Positive calcium response to 2',3'-O-(benzoyl-4-benzoyl)-ATP (BzATP) and negative response to α,β-methylene ATP suggested $P2X_2$, $P2X_4$, and $P2X_7$ as functional subunits in rat odontoblasts. Single-cell RT-PCR analysis of the cells with confirmed calcium response and immunofluorescent detection further corroborated the expression of $P2X_4$ and $P2X_7$ in odontoblasts. Overall, this study demonstrated heterogeneous expression of calcium-related ATP receptor subtypes in subsets of individual odontoblasts, suggesting extracellular ATP as a potential signal mediator for odontoblastic functions.

**10. Liu X, Yu L, Wang Q, Pelletier J, Fausther M, Sévigny J, Malmström HS, Dirksen RT, Ren YF. Expression of ecto-ATPase NTPDase2 in human dental pulp. J Dent Res. 2012 Mar;91(3):261-7.**

Dental pulpal nerve fibers express ionotropic adenosine triphosphate (ATP) receptors, suggesting that ATP signaling participates in the process of dental nociception. In this study, we investigated if the principal enzymes responsible for extracellular ATP hydrolysis, namely, nucleoside triphosphate diphosphohydrolases (NTPDases), are present in human dental pulp. Immunohistochemical and immunofluorescence experiments showed that NTPDase2 was predominantly expressed in pulpal nerve bundles, Raschkow's nerve plexus, and in the odontoblast layer. NTPDase2 was expressed in pulpal Schwann cells, with processes accompanying the nerve fibers and projecting into the odontoblast layer. Odontoblasts expressed the gap junction protein, connexin43, which can form transmembrane hemichannels for ATP release. NTPDase2 was localized close to connexin43 within the odontoblast layer. These findings provide evidence for the existence of an apparatus for ATP release and degradation in human dental pulp, consistent with the involvement of ATP signaling in the process of dentin sensitivity and dental pain.

**11. El Karim I, McCrudden MT, Linden GJ, Abdullah H, Curtis TM, McGahon M, About I, Irwin C, Lundy FT. TNF-α-induced p38MAPK activation regulates TRPA1 and TRPV4 activity in odontoblast-like cells. Am J Pathol. 2015 Nov;185(11):2994-3002.**

The transient receptor potential (TRP) channels are unique cellular sensors that are widely expressed in many neuronal and nonneuronal cells. Among the TRP family members, TRPA1 and TRPV4 are emerging as candidate mechanosensitive channels that play a pivotal role in inflammatory pain and mechanical hyperalgesia. Odontoblasts are nonneuronal cells that possess many of the features of mechanosensitive cells and mediate important defense and sensory functions. However, the effect of inflammation on the activity of the odontoblast's mechanosensitive channels remains unknown. By using immunohistochemistry and calcium microfluorimetry, we showed that odontoblast-like cells express TRPA1 and TRPV4 and that these channels were activated by hypotonicity-induced membrane stretch. Short treatment of odontoblast-like cells with tumor necrosis factor (TNF)-α enhanced TRPA1 and TRPV4 responses to their chemical agonists and membrane stretch. This enhanced channel activity was accompanied by phospho-p38 mitogen-activated protein kinase (MAPK) expression. Treatment of cells with the p38 inhibitor SB202190 reduced TNF-α effects, suggesting modulation of channel activity via p38 MAPK. In addition, TNF-α treatment also resulted in an up-regulation of TRPA1 expression but down-regulation of TRPV4. Unlike TRPV4, enhanced TRPA1 expression was also evident in

dental pulp of carious compared with noncarious teeth. SB202190 treatment significantly reduced TNF-α-induced TRPA1expression, suggesting a role for p38 MAPK signaling in modulating both the transcriptional and non-transcriptional regulation of TRP channels in odontoblasts.

**12. Maurin JC, Couble ML, Thivichon-Prince B, Magloire H. Odontoblast: a key cell involved in the perception of dentinal pain. Med Sci (Paris). 2013 Mar;29(3):293-9.**

Dentinal sensitivity is a clinical condition daily encountered by practitioners and constitutes the symptoms of dentinal hypersensitivity, a common dental pain affecting on average 30% of the population. However, the management of this pathology is not always effective due to the lack of knowledge particularly concerning the means by which dental nociceptive signals are transduced. The mechanisms underlying dentin sensitivity still remain unclear probably due to the structural and functional complexity of the players including odontoblasts, nerve endings and dentinal fluid running in the dentinal tubules. The unique spatial situation of odontoblasts, ciliated cells in close relationship with nerve terminals, suggests that they could play a pivotal role in the transduction of sensory events occurring within the dentin tissue. Our studies have identified mechano-thermosensitive transient receptor potential ion channels (TRPV1-4, TRPA8, TRPM3, KCa, TREK-1, PC1, PC2) localised on the odontoblastic membrane and at the base of the cilium. They could sense temperature variations or movements of dentinal fluid within tubules. Moreover, several voltage-gated sodium channels confer excitable properties to odontoblasts in response to injection of depolarizing currents. In vivo, these channels co-localize with nerve endings at the apical pole of odontoblasts, and their expression pattern seems to be correlated with the spatial distribution of stretch-activated KCa channels. All these data strengthen the hypothesis that odontoblasts could act as sensor cells able to transmit nociceptive signals. However, how cells sense signals and how the latter are transmitted to axons represent the main issue to be solved.

**13. Shiozaki Y, Sato M, Kimura M, Sato T, Tazaki M, Shibukawa Y. Ionotropic P2X ATP Receptor Channels Mediate Purinergic Signaling in Mouse Odontoblasts. Front Physiol. 2017 Jan 20;8:3.**

ATP modulates various functions in the dental pulp cells, such as intercellular communication and neurotransmission between odontoblasts and neurons, proliferation of dental pulp cells, and odontoblast differentiation. However, functional expression patterns and their biophysical properties of ionotropic ATP (P2X) receptors ($P2X_1$-$P2X_7$) in odontoblasts were still unclear. We examined these properties of P2X receptors in mouse odontoblasts by patch-clamp recordings. $K^+$-ATP, nonselective P2X receptor agonist, induced inward currents in odontoblasts in a concentration-dependent manner. $K^+$-ATP-induced currents were inhibited by $P2X_4$ and $P2X_7$ selective inhibitors (5-BDBD and KN62, respectively), while $P2X_1$ and $P2X_3$ inhibitors had no effects. $P2X_7$ selective agonist (BzATP) induced inward currents dose-dependently. We could not observe $P2X_{1,\ 2/3,\ 3}$ selective agonist (αβ-MeATP) induced currents. Amplitudes of $K^+$-ATP-induced current were increased in solution without extracellular $Ca^{2+}$, but decreased in $Na^+$-free extracellular solution. In the absence of both of extracellular $Na^+$ and $Ca^{2+}$, $K^+$-ATP-induced currents were completely abolished. $K^+$-ATP-induced $Na^+$ currents were inhibited by $P2X_7$ inhibitor, while the $Ca^{2+}$ currents were sensitive to $P2X_4$ inhibitor. These results indicated that odontoblasts functionally expressed $P2X_4$ and $P2X_7$ receptors, which might play an important role in detecting extracellular ATP following local dental pulp injury.

**14. Bakri MM, Yahya F, Munawar KMM, Kitagawa J, Hossain MZ. Transient receptor potential vanilloid 4 (TRPV4) expression on the nerve fibers of human dentalpulp is upregulated under inflammatory condition. Arch Oral Biol. 2018 May;89:94-98.**

Objective: Transient receptor potential vanilloid 4 (TRPV4) has been considered as a mechano-, thermo- and osmo-receptor. Under inflammatory conditions in dental pulp, teeth can become sensitive upon exposure to a variety of innocuous stimuli. The objective of the present study was to investigate the expression of the TRPV4 channel on nerve fibers in human dental pulp of non-symptomatic and symptomatic teeth associated with inflammatory conditions. Design: Dental pulp from extracted human permanent teeth was processed for fluorescence immunohistochemistry. Ten asymptomatic (normal) and 10 symptomatic (symptoms associated with pulpitis) teeth were used in this study. Nerve fibers were identified by immunostaining for a marker, protein gene product 9.5, and the cells were counterstained with 4',6-diamidino-2-phenylindole. An anti-TRPV4antibody was used to trace TRPV4 expression. Results: TRPV4 expression was co-localized with the nerve fiber marker. Immunoreactivity for TRPV4 was more intense ($p < 0.05$) in the nerves of symptomatic teeth than those of normal teeth. The number of co-localization spots was increased significantly ($p < 0.05$) in the dental pulp of symptomatic teeth compared with that of asymptomatic (normal) teeth. Conclusions: There is expression of TRPV4 channels on the nerve fibers of human dental pulp. Our findings suggest upregulation of TRPV4 expression under inflammatory conditions in the pulp. The upregulation of TRPV4 channels may be associated with the exaggerated response of dental pulp to innocuous mechanical, thermal and osmotic stimuli under inflammatory conditions.

**15. Sato M, Ogura K, Kimura M, Nishi K, Ando M, Tazaki M, Shibukawa Y. Activation of Mechanosensitive Transient Receptor Potential/Piezo Channels in OdontoblastsGenerates Acti on Potentials in Cocultured Isolectin $B_4$-negative Medium-sized TrigeminalGanglion Neurons. J Endod. 2018 Jun;44(6):984-991.e2.**

Introduction: Various stimuli to the dentin surface elicit dentinal pain by inducing dentinal fluid movement causing cellular deformation in odontoblasts. Although odontoblasts detect deformation by the activation of mechanosensitive ionic channels, it is still unclear whether odontoblasts are capable of establishing neurotransmission with myelinated A delta (Aδ) neurons. Additionally, it is still unclear whether these neurons evoke action potentials by neurotransmitters from odontoblasts to mediate sensory transduction in dentin. Thus, we investigated evoked inward currents and evoked action potentials form trigeminal ganglion (TG) neurons after odontoblast mechanical stimulation. Methods: We used patch clamp recordings to identify electrophysiological properties and record evoked responses in TG neurons. Results: We classified TG cells into small-sized and medium-sized neurons. In both types of neurons, we observed voltage-dependent inward currents. The currents from medium-sized neurons showed fast inactivation kinetics. When mechanical stimuli were applied to odontoblasts, evoked inward currents were recorded from medium-sized neurons. Antagonists for the ionotropic adenosine triphosphate receptor ($P2X_3$), transient receptor potential channel subfamilies, and Piezo1 channel significantly inhibited these inward currents. Mechanical stimulation to odontoblasts also generated action potentials in the isolectin $B_4$-negative medium-sized neurons. Action potentialsin these isolectin $B_4$-negative medium-sized neurons showed a short duration. Overall, electrophysiological properties of neurons indicate that the TG neurons with recorded evoked responses

after odontoblast mechanical stimulation were myelinated Aδ neurons. Conclusions: Odontoblasts established neurotransmission with myelinated Aδ neurons via $P2X_3$ receptor activation. The results also indicated that mechanosensitive TRP/Piezo1 channels were functionally expressed in odontoblasts. The activation of $P2X_3$ receptors induced an action potential in the Aδ neurons, underlying a sensory generation mechanism of dentinal pain.

**16. Yi X, Wang W, Xie Q. Adenosine receptors enhance the ATP-induced odontoblastic differentiation of human dental pulp cells. Biochem Biophys Res Commun. 2018 Mar 11;497(3):850-856.**

Purinergic signaling regulates various biological processes through the activation of adenosine receptors (ARs) and P2 receptors. ATP induces the odontoblastic differentiation of human dental pulp cells (HDPCs) via P2 receptors. However, there is no information available about the roles of ARs in HDPC odontoblastic differentiation induced by ATP. Here, we found that HDPCs treated with ATP showed higher activity of ADORA1 ($A_1R$), ADORA2B ($A_{2B}R$), and ADORA3 ($A_3R$). Inhibition of $A_1R$ and $A_{2B}R$ attenuated ATP-induced odontoblastic differentiation of HDPCs, whereas activation of the two receptors enhanced the odontoblastic differentiation induced by ATP. However, activation of ARs by adenosine did not induce the odontoblastic differentiation of HDPCs independently without induction of ATP. Our study indicates a positive role for ARs in ATP-induced odontoblastic differentiation of HDPCs, and demonstrates that ATP-induced odontoblastic differentiation of HDPCs may be due to the combined administration of ARs and P2 receptors. This study provides new insights into the molecular mechanisms of pulpal injury repair induced by ATP.

**17. Wang W, Yi X, Ren Y, Xie Q. Effects of Adenosine Triphosphate on Proliferation and Odontoblastic Differentiation of HumanDental Pulp Cells. J Endod. 2016 Oct;42(10):1483-9.**

Introduction: Adenosine 5'-triphosphate (ATP) is a potent signaling molecule that regulates diverse biological activities in cells. Its effectson human dental pulp cells (HDPCs) remain unknown. This study aimed to examine the effects of ATP on proliferation and differentiation of HDPCs. Methods: Reverse transcription polymerase chain reaction was performed to explore the mRNA expression of P2 receptor subtypes. Cell Counting Kit-8 test and flow cytometry analysis were used to examine the effects of ATP on proliferation and cell cycle of HDPCs. The effectsof ATP on differentiation of HDPCs were examined by using alizarin red S staining, energy-dispersive x-ray analysis, Western blot analysis, and real-time polymerase chain reaction. Results: The purinoceptors P2X3, P2X4, P2X5, P2X7, and all P2Y receptor subtypes were confirmed to present in HDPCs. ATP enhanced HDPC proliferation at 10 μmol/L concentration. However, it inhibited cell proliferation by arresting the cell cycle in G0G1 phase ($P < .05$ versus control) and induced odontoblastic differentiation, ERK/MAPK activation, and dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP) mRNA transcriptions at 800 μmol/L concentration. Suramin, an ATP receptor antagonist, inhibited ERK/MAPK activation and HDPC odontoblastic differentiation ($P < .05$ versus control). Conclusions: Extracellular ATP activates P2 receptors and downstream signaling events that induce HDPC odontogenic differentiation. Thus, ATP may promote dental pulp tissue healing and repair through P2 signaling. Results provide new insights into the molecular regulation of pulpal wound healing.

18. **Sato M, Sobhan U, Tsumura M, Kuroda H, Soya M, Masamura A, Nishiyama A, Katakura A, Ichinohe T, Tazaki M, Shibukawa Y. Hypotonic induced stretching of plasma membrane activates transient receptor potentialvanilloid channels and sodium -calcium exchangers in mouse odontoblasts. J Endod. 2013 Jun;39(6):779-87.**

Introduction: A number of transient receptor potential (TRP) channels have been identified as membrane-bound sensory proteins in odontoblasts. However, the activation properties of these channels remain to be clarified. The purpose of this study was to investigate hypotonic stimulation-induced Ca(2+) entry via TRP vanilloid subfamily member (TRPV) 1, TRPV2, and TRPV4 channels, which are sensitive to osmotic and mechanical stimuli, and their functional coupling with Na(+)-Ca(2+) exchangers (NCXs) in mouse odontoblast lineage cells. Methods: We examined TRP channel activity by measuring intracellular-free Ca(2+) concentration by using fura-2 fluorescence and ionic current recordings with whole-cell patch-clamp methods. Protein localization and messenger RNA expression were characterized using immunofluorescence and reverse-transcription polymerase chain reaction analyses. Results: Extracellular hypotonic solution-induced stretching of plasma membrane resulted in the activation of Ca(2+) influx and inward currents. TRPV1, TRPV2, and TRPV4 channel antagonists inhibited the hypotonic stimulation-induced Ca(2+) entry and currents. Their respective agonists activated Ca(2+) entry. Although the increase in the intracellular free Ca(2+) concentration decayed rapidly after the applications of these TRPV channel agonists, NCX inhibitors significantly prolonged the decay time constant. The messenger RNA expression of TRPV1, TRPV2, and TRPV4 channels; NCX isoforms 2 and 3; and dentin sialophosphoprotein were up-regulated after 24 hours of exposure to the hypotonic culture medium. Conclusions: These results indicate that stretching of the odontoblast membrane activates TRPV1-, TRPV2-, and TRPV4-mediated Ca(2+) entry, and increased intracellular-free Ca(2+) concentration is extruded via NCXs. These results suggest that odontoblasts can act as sensors that detect stimuli applied to exposed dentin and drive a number of cellular functions including dentinogenesis and/or sensory transduction.

19. **Kimura M, Sase T, Higashikawa A, Sato M, Sato T, Tazaki M, Shibukawa Y. High pH-Sensitive TRPA1 Activation in Odontoblasts Regulates Mineralization. J Dent Res. 2016 Aug;95(9):1057-64.**

Calcium hydroxide and mineral trioxide aggregate are widely used for indirect and direct pulp capping and root canal filling. Their dissociation into Ca(2+) and OH(-) in dental pulp creates an alkaline environment, which activates reparative/reactionary dentinogenesis. However, the mechanisms by which odontoblasts detect the pH of the extracellular environment remain unclear. We examined the alkali-sensitive intracellular Ca(2+) signaling pathway in rat odontoblasts. In the presence or absence of extracellular Ca(2+), application of alkaline solution increased intracellular Ca(2+) concentration, or [Ca(2+)]i Alkaline solution-induced [Ca(2+)]i increases depended on extracellular pH (8.5 to 10.5) in both the absence and the presence of extracellular Ca(2+) The amplitude was smaller in the absence than in the presence of extracellular Ca(2+) Each increase in [Ca(2+)]i, activated by pH 7.5, 8.5, or 9.5, depended on extracellular Ca(2+) concentration; the equilibrium binding constant for extracellular Ca(2+) concentration decreased as extracellular pH increased (1.04 mM at pH 7.5 to 0.11 mM at pH 9.5). Repeated applications of alkaline solution did not have a desensitizing effect on alkali-induced [Ca(2+)]i increases and inward currents. In the presence of extracellular Ca(2+), alkaline solution-induced [Ca(2+)]i increases were suppressed by application of an antagonist of transient receptor potential ankyrin subfamily member 1 (TRPA1) channels. Ca(2+) exclusion efficiency during alkaline solution-induced [Ca(2+)]i increases was reduced by a Na(+)-Ca(2+) exchanger antagonist. Alizarin red and von Kossa staining revealed increased mineralization levels under repeated high pH stimulation, whereas the TRPA1 antagonist strongly reduced this

effect. These findings indicate that alkaline stimuli-such as the alkaline environment inside dental pulp treated with calcium hydroxide or mineral trioxide aggregate-activate Ca(2+) mobilization via Ca(2+) influx mediated by TRPA1 channels and intracellular Ca(2+) release in odontoblasts. High pH-sensing mechanisms in odontoblasts are important for activating dentinogenesis induced by an alkaline environment.

**20. Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM. Odontoblast TRP channels and thermo/mechanical transmission. J Dent Res. 2009 Nov;88(11):1014-9.**

Odontoblasts function as mechanosensory receptors because of the expression of mechanosensitive channels in these cells. However, it is unclear if odontoblasts direct the signal transmission evoked by heat/cold or osmotic changes. This study investigated the effects of heat/cold or osmotic changes on calcium signaling and the functional expression of the thermo/mechanosensitive transient receptor potential (TRP) channels in primary cultured mouse odontoblastic cells, with the use of RT-PCR, fluorometric calcium imaging, and electrophysiology. TRPV1, TRPV2, TRPV3, TRPV4, and TRPM3 mRNA was expressed, but TRPM8 and TRPA1 mRNA was not. The receptor-specific stimulation of TRPV1-3 (heat-sensing receptors) and TRPV4/ TRPM3 (mechanic receptors) caused increases in the intracellular calcium concentration. Moreover, the channel activities of TRPV1-4 and TRPM3 were confirmed by a whole-cell patch-clamp technique. These results suggest that primary cultured mouse odontoblasts express heat/mechanosensitive TRP channels and play a role in the underlying mechanisms of thermo/mechanosensitive sensory transmission.

**21. Byers MR, Westenbroek RE Odontoblasts in developing, mature and ageing rat teeth have multiple phenotypes that variablyexpress all n ine voltage-gated sodium channels. Arch Oral Biol. 2011 Nov;56(11):1199-220.**

Objective: Our goal was to evaluate the expression patterns for voltage gated sodium channels in odontoblasts of developing and maturerat teeth. Design: We analysed immunoreactivity (IR) of the alpha subunit for all nine voltage gated sodium channels (Nav1.1-1.9) in teeth of immature (4 weeks), young adult (7 weeks), fully mature adult (3 months), and old rats (6-12 months). We were interested in developmental changes, crown/root differences, tetrodotoxin sensitivity or resistance, co-localization with nerve regions, occurrence in periodontium, and coincidence with other expression patterns by odontoblasts such as for transient receptor potential A1 (TRPA1). Results: We found that Nav1.1-1.9-IR each had unique odontoblast patterns in mature molars that all differed from developmental stages and from incisors. Nav1.4- and Nav1.7-IR were intense in immature odontoblasts, becoming limited to specific zones in adults. Crown odontoblasts lost Nav1.7-IR and gained Nav1.8-IR where dentine became innervated. Odontoblast staining for Nav1.1- and Nav1.5-IR increased in crown with age but decreased in roots. Nav1.9-IR was especially intense in regularly scattered odontoblasts. Two tetrodotoxin-resistant isoforms (Nav1.5, Nav1.8) had strong expression in odontoblasts near dentinal innervation zones. Nav1.6-IR was concentrated at intercusp and cervical odontoblasts in adults as was TRPA1-IR. Nav1.3-IR gradually became intense in all odontoblasts during development except where dentinal innervation was dense. Conclusions: All nine voltage-gated sodium channels could be expressed by odontoblasts, depending on intradental location and tooth maturity. Our data reveal much greater complexity and niche-specific specialization for odontoblasts than previously demonstrated, with implications for tooth sensitivity.

**22. Ricucci D, Loghin S, Lin LM, Spångberg LS, Tay FR. Is hard tissue formation in the dental pulp after the death of the primary odontoblasts a regenerative or a reparative process? J Dent. 2014 Sep;42(9):1156-70.**

Objectives: Conceptually, two types of tertiary dentine may be produced in response to caries and environmental irritations: "reactionary dentine" that is secreted by existing primary odontoblasts and "reparative dentine", formed after the death of the odontoblasts by proliferation and differentiation of progenitor cells into odontoblast-like cells. Because histologic evidence for tubular dentine generated by newly differentiated odontoblast-like cells is lacking in human teeth, the present study examined pulpal cellular changes associated with caries/restorations, in the presence or absence of pulpal exposures. Methods: Ninety-six extracted human teeth were histologically processed and serial sectioned for light microscopy: 65 contained untreated enamel/dentine caries; 20 were heavily restored and 11 had carious exposures managed by direct pulp-capping. Results: Sparsely distributed, irregularly arranged dentinal tubules were identified from the tertiary dentine formed in teeth with unexposed medium/deep caries and in restored teeth; those tubules were continuous with the tubules of secondary dentine; in some cases, tubules were absent. The palisade odontoblast layer was reduced to a single layer of flattened cells. In direct pulp-capping of pulp exposures, the defects were repaired by the deposition of an amorphous dystrophic calcified tissue that resembled pulp stones more than dentine, sometimes entrapping pulpal remnants. This atubular hard tissue was lined by fibroblasts and collagen fibrils. Conclusions: Histological evidence from the present study indicates that reparative dentinogenesis cannot be considered as a regenerative process since the so-formed hard tissue lacks tubular features characteristic of genuine dentine. Rather, this process represents a repair response that produces calcified scar tissues by pulpal fibroblasts. Clinical significance: Formation of hard tissue in the dental pulp after the death of the primary odontoblasts has often been regarded by clinicians as regeneration of dentine. If the objective of the clinical procedures involved is to induce healing, reduce dentine hypersensitivity, or minimise future bacteria exposure, such procedures may be regarded as clinical success. However, current clinical treatment procedures are not adept at regenerating physiological dentne because the tissues formed in the dental pulp are more likely the result of repair responses via the formation of calcified scar tissues.

## 4. Selección final de artículos por temática

### *4.1 Tipos de estudios participantes (criterios de inclusión)*

Los artículos incluidos en este estudio fueron considerados elegibles si cumplían las siguientes características: a) Estudios que evalúen la función del odontoblasto en la nocicepción y/o sensibilidad dental, b) Estudios que evalúen la función del odontoblasto en la inmunidad pulpar, c) Estudios que evalúen el papel del odontoblasto en la inflamación neurogénica en la pulpa dental, d) Estudios en idioma Inglés, e) Estudios publicados en bases de datos como

MEDLINE, EMBASE, Lilacs y Science Direct, f) Estudios de reivisión de la literatura y ensayos clínicos, g) Estudios publicados entre enero de 2009 a junio de 2018.

*4.2 Tipos de estudios participantes (criterios de exclusión)*

Los artículos excluidos en este estudio no fueron considerados elegibles si cumplían las siguientes características: a) Estudios donde se evalúen la función del odontoblasto como célula secretora de sustancia biomineralizada, b) Estudios que evalúen agentes medicamentosos como inductores de diferenciación de células madres en linaje de odontoblastos.

**5. Proceso de extracción de información de artículos por temática**

Inicialmente, una revisora (HBRP) examinó de forma independiente los títulos, los resúmenes y textos completos de los artículos arrojados por la búsqueda. La revisora no estaba cegada a los autores, su procedencia, y sitio de publicación. Se obtuvo el informe completo de todos los estudios que aparentemente cumplían los criterios de inclusión o de los que no aportaban suficiente información con solo el título y las palabras claves con el fin de tomar una decisión clara. La autora de la revisión evaluó de forma independiente todos los estudios. Los estudios que cumplieron los criterios de inclusión y exclusión fueron seleccionados para le realización de este estudio.

**6. Proceso estructuración de artículo**

Se realizó una búsqueda electrónica de la literatura en bases de datos como MEDLINE, EMBASE, Lilacs y Science Direct. Para la búsqueda se utilizaron encabezados de términos médicos (MeSH), descriptores en ciencias de la salud (DeCS) y palabras claves. Se utilizaron los operadores booleanos OR, AND.

La búsqueda comprendió artículos publicados en revistas indexadas con fechas desde enero

de 2009 hasta junio de 2018. La estrategia de búsqueda completa se estableció para cada base de datos consultada, sobre la estrategia de búsqueda desarrollada para MEDLINE.

## 7. Proceso de Edición en inglés y en español para publicación

Una vez terminado el artículo, se realizará corrección de estilo y traducción al inglés por parte de un editor certificado en español y en inglés para este fin teniendo como base las normas internacionales de Vancouver.

## 10. Sustento legal

- ✓ Los autores declaran su responsabilidad de solicitar los permisos correspondientes por el uso de derechos tanto de material impreso o electrónico, y se responsabilizan por el pago de cualquier gravamen relacionado con el uso de estos permisos.
- ✓ Adicionalmente declaran que no tienen intereses económicos relacionados con el presente trabajo que pueda crear cualquier conflicto de intereses.

# 11. Resultados

## 1. Resumen de proceso de búsqueda de información

La búsqueda en la base de datos electrónica, actualizada por última vez el 25 de Junio de 2018 arrojó 492.157 publicaciones, de las cuales 0 se encontraron en MEDLINE, 160 en EMBASE, 147 en Science Direct y 27 en Lilacs. Después de la evaluación de los títulos y los resúmenes se eliminaron inmediatamente 521 artículos. Los 150 artículos restantes fueron examinados y excluidos si no cumplían con los criterios de inclusión o se encontraban duplicados dando como resultado un total de 93 artículos aceptados para la revisión final y procesados para la extracción de datos.

### Métodos

Con el fin de estructurar de manera metódica la búsqueda de información se realizó el siguiente proceso:

1) Se definieron las siguientes variables: Complejo dentino-pulpar, fisiología de los odontoblastos, nocicepción, inmunología, inflamación neurogénica.
2) Se utilizaron las siguientes palabras clave: Odontoblasto, nocicepción, inmunología, inflamación.
3) Se buscaron los siguientes tipos de estudios: ensayos clínicos aleatorizados, revisiones de la literatura.
4) Se tuvieron en cuenta los siguientes criterios de inclusión: artículos publicados entre 2009-2018, idioma inglés.
5) Se utilizaron las siguientes estrategias de búsquedas:

| | |
|---|---|
| #1 | Odontoblast |
| #2 | Odontoblast AND nociception OR sensibility |
| #3 | Odontoblast AND immunology OR immunology AND inflammation |

| | |
|---|---|
| #4 | Odontoblast AND nociception OR sensibility |
| # 5 | Odontoblast AND nociception OR odontoblasts AND immunology OR odontoblast AND inflammation |

2. **Resultados de proceso de extracción de información**

## DESARROLLO DEL TEMA

Los odontoblastos están encargados de la formación de la dentina en la superficie más externa de la pulpa dental. En la actualidad con un elevado incremento en la evidencia científica que en los últimos años ha sugerido que los odontoblastos también funcionan como células sensoriales o inmunes.

*Odontoblastos y Respuesta Sensorial-Nocicepción:*

Como se sabe el odontoblasto expresa canales dependientes de voltaje de Na+ (NaV) estos son importantes para la regulación de la función celular (26, 27). Esta una glicoproteína de transmembrana y forma un poro con una subunidad $\alpha$ y tiene una subunidad auxiliar $\beta$. Se han identificado la expresión de 9 isoformas de canales en ratones, que su localización a nivel coronal y/o radicular dependen del grado de la maduración dental (28, 29). Según Bleicher en el 2013, una subunidad es la NaV 1, la cual se expresa en ratones en donde se subdivide en NaV 1.7 y NaV 1.8, expresándose a nivel del sistema nervioso periférico y ambos son importantes para la transmisión normal del dolor; a nivel de la corona dental hay ausencia de este canal y el NaV 1.8 está presente en la dentina altamente inervada (30, 31).

Por otra parte, Bayers *et al*., en el 2011, refieren que el NaV1.3 va incrementando su intensidad durante el desarrollo de los odontoblastos, excepto donde la inervación dentinal es más densa (Sato *et al*., 2013) (32, 32). El NaV1.6 está localizado a nivel de la zona cuspídea y cervical en odontoblastos maduros. NaV1.9 aumenta su expresión en dientes con procesos de pulpitis

sintomática. (33, 34). La deformación del cuerpo del odontoblasto, puede ser provocada por el movimiento del fluido dentinal por la previa estimulación de la dentina, activando las concentraciones de $Ca^{2+}$ intracelular, conllevando a la despolarización (Tsumura *et al*., 2010; Ichikawa *et al*., 2012; Kojima *et al*., 2015). Se sugiere que los canales de $Na^{+}$ en odontoblastos humanos tienen un papel importante en modular las funciones celulares, por ejemplo no solo para la estabilización del potencial de membrana, sino también durante procesos de dentinogénesis en entornos fisiológicos por el aumento en la apertura de los canales de Na+ (35, 36).

En los odontoblastos se encuentran presentes los canales permeables de calcio el TRP (Receptor Potencial Transitorio), constituyen un familia grande de proteínas transmembranas, involucradas en diversas funciones celulares como la aposición y resorción ósea, transducción sensorial en respuesta a cambios térmicos, tacto-presión y cambios en el pH (Son *et al*., 2009; Wetsel *et al*., 2017; Sato *et al*., 2018) (17, 37, 38).

En mamíferos, los TRP se han clasificado en 6 subfamilias sobre la base de homología de secuencia de aminoácidos: TRPC (canónica), TRPV (vaniloide), TRPM (melastatina), TRPP (policistina), TRPML (mucolipin), TRPA (ankirina) (39).

El TRPV1, el cual es sensible al calor a >42 °C, pH de 7.4, metabolitos de la lipoxigenasa, protones y capsaicina, canabinoides en donde tiene un papel importante para modular funciones fisiopatológicas y la subsecuente secreción de dentina terciaria; es decir actúan como sensores moleculares (Tsumura *et al*., 2013; Sato *et al*., 2013; Egbuniwe *et al*., 2014; Kimura *et al*., 2016) (40, 41).

Que *et al*., en el 2017 evalúan los canales TRP que son activados por cannabinoides (CB) a concentraciones muy elevadas mediante unos receptores:  el CB1 está presente a nivel del sistema nervioso central y en algunos tejidos periféricos como la glándula pituitaria, células inmunes, tejidos reproductivos, tejidos gastro-intestinales, corazón, pulmón, vejiga y glándula suprarrenal, así mismo el receptor CB2, se expresa a nivel de las células B y las Natural Killer.

La vía cAMP mediada por el receptor CB1 y el TRPV1, modula la dentinogénesis y la homeóstasis celular (42, 43).

El TRPV2, se activa frente a un estímulo nocivo de calor. Bevan *et al*; en el 2009, el TRPV4 tiene función mecano-sensitiva y osmoreceptora; así mismo Bakri *et al.,* en el 2018, sugieren que la expresión del TRPV4 aumenta a nivel de las fibras nerviosas de pulpa dental durante la inflamación (44, 45).

Se ha identificado la expresión del TRPM7 a nivel del proceso odontoblástico y su rol tanto en la fase de la biomineralización por medio de la regulación intracelular del Mg2+ y la función de la ALP, la cual actúa como nociceptor a estímulos externos y mecano-receptor para el proceso de biomineralización (46, 57). Farges *et al*., en el 2013 refieren que la expresión del TRPM8 en los odontoblastos sugiere que estas células actúan como receptores para la estimulación por frío no nociceptivo a <22ºC (39). Aunque han referido que los odontoblastos liberan ATP, el cual es un neurotransmisor, se sugiere que estas células tienen la capacidad de transmitir señales a las fibras nerviosas circundantes (Liu *et al.,* 2015; Shibukawa *et al*., 2015; Nishiyama *et al*., 2016) (40).

Kimura *et al*,. en 2016, refiere que el TRPA1 es un canal iónico polimodal que se puede activar por medio de las alquilamidas como hidróxido sanshool y componentes endógenos como: peróxido de hidrógeno, ácido nitrico-oleico, ácidos epoxieicosatrienoicos (5,6-ETT; 8,9-ETT). También los canales TRPA1 pueden ser activados por modificaciones covalentes de cisteína y lisina en el extremo N-terminal (24, 54).

Se han identificado 6 proteínas que codifican para 4 genes de ASICs los cuales son: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 y ASIC4; tienen funciones mecano-sensitivas, quimiosensitivas y nociceptivas. En donde a nivel del las neuronas trigeminales murinas se han detectado 17 de los 28 genes de canales TRP como: TRPA1, TRPC1, TRPC3,TRPC4, TRPC5, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7,TRPM8, TRPV1, TRPV2,

TRPV4, TRPML1 y TRPP2; en el ganglio trigeminal humano se han identificado 10 TRP como: TRPC1, TRPM2, TRPM3, TRPM7, TRPM8, TRPV1, TRPV2, TRPV3,TRPV4 and TRPML1) y ASIC1-3. (18,19, 41).

Se sugiere que sí puede ser posible la cercanía que hay entre la empalizada odontoblástica y la porción terminal de las fibras nerviosas, cuando se estimula el odontoblasto se da la liberación de mediadores al interior de los gaps que hay entre los odontoblastos y la fibra nerviosa, seguido de la producción de una señal aferente (28).

Se han propuesto diferentes tipos de mediadores (Liu et., 2012; Bond et., 2013; Lee *et al*., 2017) como la galanina, glutamato, óxido nítrico, purinas extracelulares como el Adenosin y el ATP mediante la transmisión purinérgica (29, 30). Esta transmisión purinérgica consiste en que las purinas y pirimidinas median la respuesta celular por la estimulación de los receptores específicos; en donde cada célula tiene una reserva de ATP que bajo condiciones fisiológicas es liberado por hemicanales connexin 43, pannexin-1, canales de aniones y mecanismos dependientes de Ca2+.(31).

En cuanto a los receptores purinérgicos estos se dividen en dos categorías: receptores P1 y P2 (Bond *et al.,* 2013). El P1 son receptores para adenosina, acoplados a la proteína G, se subclasifican en receptores P2Y y los receptores P2 son para el ATP y se subclasifican en canales catiónicos ionotrópicos P2X. Actualmente se han identificado en mamíferos siete receptores P2X (1R-7R) y ocho receptores P2Y (P2Y1R, P2Y2R, P2Y4R, P2Y6R, P2Y11R, P2Y12R, P2Y13R y P2Y14R) (Bond *et al.,* 2013; Shibukawa *et al.,* 2015; Le Fur-Bonnabesse *et al.,* 2017). Los P2XR se expresan tanto en las células nociceptivas del ganglio trigeminal y en la pulpa dental (Fu *et al*., 2015; Iwamoto *et al*., 2017) (32).

Por otra parte, se detectaron fibras nerviosas positivas a receptores P2X mielinizadas y no mielinizadas a nivel del plexo subodontoblástico en estrecha cercanía con los odontoblastos. Además, los odontoblastos expresan diferentes subtipos de P2XR (Lee *et al*., 2017; Shiozaki

*et al*., 2017) lo que sugiere que el ATP podría regular la función fisiológica de los odontoblastos (Iwamoto *et al*., 2017). También, los subtipos de receptores P2Y están presentes en células de pulpa (Wang *et al.,* 2016), neuronas del ganglio trigeminal (Li *et al*., 2014; Kawaguchi *et al.,* 2015), así como en los odontoblastos (Sato *et al.,* 2015; Wanget *et al.,* 2016; Nishiyama *et al*., 2016) (41, 42).

Por los estudios recientes acerca del odontoblasto, se ha determinado que el estímulo mecánico o la estimulación directa de los odontoblastos conducen a la liberación de ATP, presumiblemente a través de canales iónicos (43). Por ejemplo, la activación del TRPA1 y TRPV4, pero no del receptor TRPV1, en células humanas similares a odontoblastos produce un incremento en la liberación de ATP la cual se puede inhibir mediante los antagonistas selectivos de TRP (Ikeda *et al.,* 2013; Khatibi *et al.,* 2015; Le Fur-Bonnabesse *et al*., 2017) (44, 45, 53).

Por otra parte, ya se ha evidenciado que los odontoblastos constituyen una barrera importante contra la infección bacteriana (caries dental). En donde la supervivencia de estas células son fundamentales para el pronóstico de la pulpa inflamada. (Li *et al.,* 2018) sugiere que la autofagia es fundamental para la respuesta antiinflamatoria por parte de los odontoblastos, teniendo en cuenta que la autofagia se conserva evolutivamente reciclando proteínas de larga longevidad junto con organelos lesionados para poder generar una homeostasis a nivel intracelular (68, 69).

Zhang *et al.* en 2018, reportan que la progresión de la autofagia incluye la formación de vesículas de doble membrana que pueden expandirse en autofagosomas y este proceso es importante tanto en la regulación fisiológica como patológica como en el cáncer, la neurodegeneración e inflamación (70). La autofagia también participa a nivel del desarrollo dental y el envejecimiento de los odontoblastos (Couve *et al*., 2011; Couve *et al*., 2012; Takanchea *et al*., 2018 ; Li *et al.,* 2018; Zhang *et al*., 2018) (71, 72, 73, 74)

Li *et al*., mencionan que los mecanismos de la regulación de la autofagia son complejos y que a su vez hay muchos factores transcripcionales como la familia FoxO, TFEB y el CLOCK los

cuales regulan la autofagia en respuesta a diferentes estímulos tanto intra como extracelulares. Pero los factores de transcripción específicos responsables de activar e inducir por el proceso inflamatorio en los odontoblastos no han sido identificados hasta la actualidad (68).
En donde la familia del FoxO está conformado por: el FoxO1, FoxO3a, FoxO4 y las subfamilias FoxO6 en células de mamíferos. Sin embargo el FoxO3a ha sido destacado debido a su regulación de la inmunidad, longevidad, metabolismo, diferenciación y apoptosis celular. El FoxO3a puede ser fosforilado e inhibido por PI3K/Akt y la disminución de este factor en células tumorales promueve la proliferación, conllevando al cáncer; también se ha encontrado que tiene función pro-apoptótica, optimizando las funciones protectoras de la autofagia frente al estrés ambiental (68, 70). Los odontoblastos pueden usar FoxO3a como un sensor para detectar el estrés celular (68).

La activación de Akt puede desencadenar varias respuestas biológicas, su fosforilación reduce la activación transcripcional de FoxO3a por la inhibición de la traslocación de FoxO3a al núcleo celular. Li *et al*., en el 2018, reportaron cuando el FoxO3 fue activado y se dio la traslocación del núcleo, se observó un aumento de los marcadores de la autofagia en estadios iniciales; sugiriendo que la autofagia podría ser provocada por FoxO3a para proteger las células en el estadío inicial. En estadios avanzados, la autofagia va disminuyendo, pero FoxO3a va aumentando y el p-Akt fue regulado positivamente, planteando que el Akt está implicado en la protección contra la muerte celular e inflamación en lugar de FoxO3a. Por lo tanto se propone que el FoxO3a tiene como función la regulación de la activación de la autofagia para la supervivencia de los odontoblastos (68).

Los canales nociceptivos de TRP participan en un múltiples modalidades de percepción del dolor como el dolor inflamatorio, dolor neuropático, dolor visceral y dolor relacionado bajo condiciones patológicas como el cáncer y migraña (60).

Por ejemplo, la participación de TRPV1 a nivel del dolor inflamatorio es el más expresado entre los canales de TRP. Sin embargo, los antagonistas de TRPV1 son eficientes disminuyendo la hiperalgesia térmica bajo condiciones inflamatorias y aumentando la percepción del dolor urente nociceptivo (61). Además, la evidencia científica respalda el papel del TRPV1 y TRPA1

en la pulpitis sintomática, sugiriendo que median la hiperalgesia tanto al frío como al estímulo mecánico (62).

Por otra parte, el óxido nítrico (NO) tiene una participación en el proceso inflamatorio el cual es sintetizado y expresado por enzimas neuronales y endoteliales (e) NO sintasas (NOS) o por la enzima inducible (i) expresada en iNOS. En respuesta frente a estímulos fisiológicos se desencadena una señal de Ca2 +a nivel intracelular a su vez activando el nNOS y eNOS (Korkmaz *et al*., 2010). Pero en las células en reposo el iNOS no se expresa, pero está regulado a nivel de los genes por mediadores inflamatorios, como lipopolisacáridos bacterianos (LPS) o citoquinas proinflamatorias como la interluquina IL-1, TNF-α, IFN-γ.

En concentraciones fisiológicamente bajas, (Korkmaz *et al.,* 2011) el NO es producido por nNOS y eNOS e interactúa directamente con el hierro y así activar la enzima para producir la molécula efectora intracelular de guanosina cíclica 3 ', 5'-monofosfato (cGMP); en donde la formación de dicha molécula cGMP a su vez desencadenará una gran variedad de respuestas celulares como: proliferación y diferenciación celular, neurotransmisión y vasodilatación (65, 66).

En cuanto a la enzima soluble de gunilato ciclasa de No (sGC), es una enzima heterodimérica que consiste en unas subunidades α y β, y la expresión de ambas subunidades se requiere para la actividad catalítica de la enzima, se han identificado la existencia de las subunidades α2 y β1 de sGC y formación dependiente de NO de cGMP en odontoblastos (Korkmaz *et al.,* 2011). También se ha encontrado que la inflamación del complejo dentino-pulpar induce una disminución a los niveles de proteína α1-, β1- y de la subunidad α2 de sGC en los odontoblastos. En donde el proceso inflamatorio se da la producción de especies reactivas de oxígeno (ROS), de especies reactivas de nitrógeno (RNS) y de los mediadores inflamatorios; los cuales pueden estar involucrados en la reducción de las subunidades α1, β1 y α2 del sGC en los odontoblastos. Por lo tanto se sugiere que el sGC es una enzima crítica para modular los diferentes efectos biológicos del NO en odontoblastos sanos (65, 72).

Song *et al.,* 2017 refieren que el pannexin desempeñan un rol crucial en la modulación de la homeostasis tisular y la regulación de la patogénesis de los diferentes estados inflamatorios. Se sugiere que el Panx3 contribuye con interacción entre las células inmunes y musculares, participa en diferentes vías de señalización intracelular modulando la expresión génica y actividad celular (74). Además, Song *et al*., en el 2017 evaluaron que el Panx3 se manifiesta en el tejido pulpar y en las células odontoblásticas humanas, planteando una hipótesis de que únicamente el Panx3 desempeña un papel fundamental durante el tratamiento de la inflamación pulpar, pero no hay estudios que confirmen esta hipótesis actualmente. He informó que el Panx3 regula la expresión de citoquinas proinflamatorias como IL-1b e IL-6 en respuesta a la estimulación de TNF-α mediante la vía de señalización NF-κB. (77, 78).

*Odontoblastos y respuesta Inmune:*

Para que se de una respuesta inmunulógica competente y adecuada frente a la invasión microbiana, se debe de tener una célula especializada la cual detecte rápidamente los patógenos con unos receptores tanto extracelulares como intracelulares.

Los NLR y TLR tienen como función el reconocimiento de una gran variedad de molécuas asociadas a patógenos (PAMPs) (79). Los TLR son unos receptores los que están anclados a nivel de la membrana celular, actúan a nivel extracelular, representan la primera línea de defensa y hasta la actualidad se han descrito diez TLR humanos por ejemplo, y el TLR4 reconoce los componentes de las bacterias Gram-negativas como el lipopolisacárido (LPS) (80). Por otro lado, los NLR actúan a nivel intracelular y representan una segunda línea de defensa contra los patógenos que evaden la superficie celular (81).

Las células tipo odontoblasto expresan genes TLR1, TLR6, TLR9 (Farges *et al*., 2009; Staquet *et al.,* 2011; Jang *et al.,* 2015).; sugiriendo que el odontoblasto tiene la capacidad de reconocer una variedad de PAMPs como: lipopéptidos diacetilados (TLR2, TLR6), RNA viral (TLR3), Flagelina (TLR5), LPS (TLR4), lipopéptidos triacetilados (TLR1,TLR2) (82, 83).

Según Yumoto *et al.,* 2018, por medio estudios con microarray de DNA y PCR reporta que el TLR3, TLR7, TLR8 se han detectado en odontoblastos y en el tejido pulpar. En donde el TLR8 incrementa su expresión en los odontoblastos; sugiriendo que este puede reconocer RNA viral participando así de la respuesta inmune innata a nivel del complejo dentino-pulpar (84, 85).
Los odontoblastos expresan la familia de proteínas NRL como el NOD1 el cual lo expresan los tejidos pulpares humanos frente a un proceso infeccioso o sin este, a nivel del citoplasma de los odontoblastos y de células del endotelio vascular, pero si hay un proceso infeccioso en curso se expreso el NOD1 en la capa odontoblástica radicular (86, 87).

También expresan los odontoblastos unos péptidos las cuales son las beta-defensinas (hBD), cuya función es la disrupción de la integridad de la membrana plasmática del microorganismo. Se ha evidenciado la expresion de hBD-1 y hBD-4 en los dientes sanos (88, 89). El hBD-1 se expresa en niveles minimos en el citoplasma del odontoblasto y el hBD-2 es indetectable, pero si hay una detección del LPS a nivel de estudios se sugiere que se expresa el hBD-2 (90, 91).

3. **Artículo original con su bibliografía**

## LA OTRA VISIÓN DEL ODONTOBLASTO. UNA REVISIÓN NARRATIVA

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*RESUMEN*

**Antecedentes:** El odontoblasto durante muchos años ha sido descrito como una célula especializada encargada de secretar la matriz extracelular que favorece la biomineralización del complejo pulpo-dentinal, pero en la actualidad las diferentes investigaciones han demostrado que esta célula de origen ectomesenquimal cumple múltiples funciones

reguladoras en los procesos fisiopatológicos del complejo dentino-pulpar. **Objetivo:** Revisar y evidenciar las múltiples funciones del odontoblasto a nivel inmunológico, vascular y neuronal como modulador de las respuestas celulares y moleculares en la regeneración y/o reparación en el complejo pulpo-dentinal. **Metodología:** Se realizó una búsqueda electrónica en bases de datos desde enero de 2009 hasta junio de 2018. Las bases de datos que se utilizaron fueron: PUBMED, EMBASE, MEDLINE, Lilacs, Sciente Direct y se incluyeron artículos en Inglés. **Resultados:** La búsqueda en la base de datos electrónica arrojo 492.157 publicaciones. Después de la evaluación de los títulos, resumen, criterios de inclusión y exclusión se seleccionaron un total de 94 artículos para la revisión. **Conclusiones:** Este estudio examinó y comparó la literatura disponible actualmente acerca de las múltiples funciones del odontoblasto como la nocicepción, la respuesta sensorial a múltiples estímulos externos, la capacidad inmunológica y la partipación en procesos inflamatorios, encontrando que esta célula especializada tiene la capacidad de participar y modular los procesos fisiopatológicos tanto de la dentina como de la pulpa.

*INTRODUCCIÓN*

Cada tejido se compone de múltiples tipos de células que son evolutivas y funcionalmente integradas en la unidad que llamamos un órgano (1). El complejo dentino-pulpar, es un sistema complejo y completo en donde el órgano del diente requiere de un sistema vásculo-nervioso que favorezca la viabilidad celular que conforma dicho sistema, mediante el transporte de oxígeno, nutrientes, entre otros; y la parte sensorial, mediante las fibras nerviosas y el odontoblasto para la propicepción y modulación de las fuerzas masticatorias; por lo tanto, este complejo tiene la propiedad mecano-sensorial (2). En donde diversas células pulpares, el sistema inmune, el sistema vascular y la inervación participan conjuntamente, para conformar el complejo dentinopulpar en un un órgano funcional que puede detectarse y protegerse (3).

El tejido pulpar está rodeado y protegido de tejido biomineralizado, cuyas estructuras duras son el esmalte y la dentina, en donde si uno de estos tejidos biomineralizados se llegaran a lesionar, bien sea por un proceso infeccioso y/o trauma dento-alveolar, el complejo dentino

pulpar tiene un rol importante el cual es inducir a la aposición de dentina terciaria, la cual será la dentina reaccionaria mediante el estímulo de una célula multifuncional: el odontoblasto, en donde una de sus funciones es la secreción de dentina y así proteger la pulpa dental (4, 5).

Los odontoblastos (Sasaki et. al., 1996), son células post mitóticas de gran longevidad, cuyo origen se da a partir de las células de la cresta neural craneal, y su ubicación está a nivel del límite entre la dentina y la pulpa, junto con el rol en la aposición de tejido biomineralizado durante toda la vida del diente. Los odontoblastos son similares a las neuronas y los cardiomiocitos, los cuales no pueden ser reemplazados (6, 7).

En la estructura de su morfología, presentan procesos citoplasmáticos los cuales se extienden hacia los túbulos dentinales formando una sola capa de cuerpos columnares y altamente polarizados, cuando están en estadío maduro presentan retículo endoplasmático liso y rugoso, aparato de golgi y mitocondrias para poder sintetizar proteína relacionadas con la dentinogénesis, también presentan uniones gap, las cuales son principalmente las uniones connexin, podrían estar involucrado en la transducción de señales autocrinas y paracrinas (Fried et. al., 1996) (8, 9).

En su fase madura, los odontoblastos expresan ciertos canales iónicos como TRPV, TRPA, lo que sugiere que pueden estar relacionados con una función sensorial (Chung *et al*., 2013). Esto podría lograrse a través de comunicaciones con fibras nerviosas mediante la liberación de ATP y/o a través de interacciones con células inmunes (10, 11).

El odontoblasto durante muchos años ha sido descrito como una célula especializada encargada de secretar la matriz extracelular que favorece la biomineralización del complejo pulpo-dentinal, pero en la actualidad las diferentes investigaciones han demostrado que esta célula de origen ectomesenquimal cumple múltiples funciones reguladoras en los procesos

fisiopatológicos a nivel nociceptivo, inmunológico y vascular; participando activamente en las fases de regeneración y/o reparación pulpo-dentinal (12, 13) .

Por lo tanto, este abordaje permite al profesional tener una nueva y amplia perspectiva acerca del odontoblasto y así enfocar diversas terapéuticas clínicas alternativas en el campo de la endodoncia, favoreciendo los procesos de regeneración y/o reparación tisular para el complejo pulpo-dentinal.

El objetivo de este estudio es revisar y evidenciar las múltiples funciones del odontoblasto a nivel inmunológico, vascular y neuronal como modulador de las respuestas celulares y moleculares en la regeneración y/o reparación en el complejo pulpo-dentinal.

## *MATERIALES Y MÉTODOS*

Este estudio de revisión narrativa de la literatura se ha estructurado en acuerdo con la lista de chequeo de revisiones.

### *Tipos de estudios participantes (criterios de inclusión)*

Los artículos incluidos en este estudio fueron considerados elegibles si cumplían las siguientes características: a) Estudios que evalúen la función del odontoblasto en la nocicepción y/o sensibilidad dental, b) Estudios que evalúen la función del odontoblasto en la inmunidad pulpar, c) Estudios que evalúen el papel del odontoblasto en la inflamación neurogénica en la pulpa dental, d) Estudios en idioma Inglés, e) Estudios publicados en bases de datos como MEDLINE, EMBASE, Lilacs y Science Direct, f) Estudios de reivisión de la literatura e in vitro, g) Estudios publicados entre enero de 2009 a junio de 2018.

*Tipos de estudios participantes (criterios de exclusión)*

Los artículos excluidos en este estudio no fueron considerados elegibles si cumplían las siguientes características: a) Estudios donde se evalúen la función del odontoblasto como célula secretora de sustancia biomineralizada, b) Estudios que evalúen agentes medicamentosos como inductores de diferenciación de células madres en linaje de odontoblastos.

*Proceso de extracción de información de artículos por temática*

Inicialmente, una revisora (HBRP) examinó de forma independiente los títulos, los resúmenes y textos completos de los artículos arrojados por la búsqueda. La revisora no estaba cegada a los autores, su procedencia, y sitio de publicación. Se obtuvo el informe completo de todos los estudios que aparentemente cumplían los criterios de inclusión o de los que no aportaban suficiente información con solo el título y las palabras claves con el fin de tomar una decisión clara. La autora de la revisión evaluó de forma independiente todos los estudios. Los estudios que cumplieron los criterios de inclusión y exclusión fueron seleccionados para le realización de este artículo.

*Proceso estructuración de artículo*

Se realizó una búsqueda electrónica de la literatura en bases de datos como MEDLINE, EMBASE, Lilacs y Science Direct. Para la búsqueda se utilizaron encabezados de términos médicos (MeSH), descriptores en ciencias de la salud (DeCS) y palabras claves. Se utilizaron los operadores booleanos OR, AND.

La búsqueda comprendió artículos publicados en revistas indexadas con fechas desde enero de 2009 hasta junio de 2018. La estrategia de búsqueda completa se estableció para cada base de datos consultada, sobre la estrategia de búsqueda desarrollada para PUBMED.

*DESARROLLO DEL TEMA*

Los odontoblastos están encargados de la formación de la dentina en la superficie más externa de la pulpa dental. En la actualidad con un elevado incremento en la evidencia científica que en los últimos años ha sugerido que los odontoblastos también funcionan como células sensoriales o inmunes.

*Odontoblastos y Respuesta Sensorial-Nocicepción:*

Como se sabe el odontoblasto expresa canales dependientes de voltaje de Na+ (NaV) estos son importantes para la regulación de la función celular (26, 27). Esta una glicoproteína de transmembrana y forma un poro con una subunidad $\alpha$ y tiene una subunidad auxiliar $\beta$. Se han identificado la expresión de 9 isoformas de canales en ratones, que su localización a nivel coronal y/o radicular dependen del grado de la maduración dental (28, 29). Según Bleicher en el 2013, una subunidad es la NaV 1, la cual se expresa en ratones en donde se subdivide en NaV 1.7 y NaV 1.8, expresándose a nivel del sistema nervioso periférico y ambos son importantes para la transmisión normal del dolor; a nivel de la corona dental hay ausencia de este canal y el NaV 1.8 está presente en la dentina altamente inervada (30, 31).

Por otra parte, Bayers *et al*., en el 2011, refieren que el NaV1.3 va incrementando su intensidad durante el desarrollo de los odontoblastos, excepto donde la inervación dentinal es más densa (Sato *et al*., 2013) (32, 32). El NaV1.6 está localizado a nivel de la zona cuspídea y cervical en odontoblastos maduros. NaV1.9 aumenta su expresión en dientes con procesos de pulpitis sintomática. (33, 34). La deformación del cuerpo del odontoblasto, puede ser provocada por el movimiento del fluido dentinal por la previa estimulación de la dentina, activando las concentraciones de $Ca^{2+}$ intracelular, conllevando a la despolarización (Tsumura *et al.,* 2010; Ichikawa *et al.,* 2012; Kojima *et al.,* 2015). Se sugiere que los canales de $Na^{+}$ en odontoblastos humanos tienen un papel importante en modular las funciones celulares, por ejemplo no solo

para la estabilización del potencial de membrana, sino también durante procesos de dentinogénesis en entornos fisiológicos por el aumento en la apertura de los canales de Na+ (35, 36).

En los odontoblastos se encuentran presentes los canales permeables de calcio el TRP (Receptor Potencial Transitorio), constituyen un familia grande de proteínas transmembranas, involucradas en diversas funciones celulares como la aposición y resorción ósea, transducción sensorial en respuesta a cambios térmicos, tacto-presión y cambios en el pH (Son *et al.,* 2009; Wetsel *et al.,* 2017; Sato *et al.,* 2018) (17, 37, 38).

En mamíferos, los TRP se han clasificado en 6 subfamilias sobre la base de homología de secuencia de aminoácidos: TRPC (canónica), TRPV (vaniloide), TRPM (melastatina), TRPP (policistina), TRPML (mucolipin), TRPA (ankirina) (39).

El TRPV1, el cual es sensible al calor a >42 °C, pH de 7.4, metabolitos de la lipoxigenasa, protones y capsaicina, canabinoides en donde tiene un papel importante para modular funciones fisiopatológicas y la subsecuente secreción de dentina terciaria; es decir actúan como sensores moleculares (Tsumura *et al.,* 2013; Sato *et al*., 2013; Egbuniwe *et al.,* 2014; Kimura *et al*., 2016) (40, 41).

Que *et al*., en el 2017 evalúan los canales TRP que son activados por cannabinoides (CB) a concentraciones muy elevadas mediante unos receptores:  el CB1 está presente a nivel del sistema nervioso central y en algunos tejidos periféricos como la glándula pituitaria, células inmunes, tejidos reproductivos, tejidos gastro-intestinales, corazón, pulmón, vejiga y glándula suprarrenal, así mismo el receptor CB2, se expresa a nivel de las células B y las Natural Killer. La vía cAMP mediada por el receptor CB1 y el TRPV1, modula la dentinogénesis y la homeóstasis celular (42, 43).

El TRPV2, se activa frente a un estímulo nocivo de calor. Bevan *et al*; en el 2009, el TRPV4 tiene función mecano-sensitiva y osmoreceptora; así mismo Bakri *et al.,* en el 2018, sugieren

que la expresión del TRPV4 aumenta a nivel de las fibras nerviosas de pulpa dental durante la inflamación (44, 45).

Se ha identificado la expresión del TRPM7 a nivel del proceso odontoblástico y su rol tanto en la fase de la biomineralización por medio de la regulación intracelular del Mg2+ y la función de la ALP, la cual actúa como nociceptor a estímulos externos y mecano-receptor para el proceso de biomineralización (46, 57). Farges *et al*., en el 2013 refieren que la expresión del TRPM8 en los odontoblastos sugiere que estas células actúan como receptores para la estimulación por frío no nociceptivo a <22ºC (39). Aunque han referido que los odontoblastos liberan ATP, el cual es un neurotransmisor, se sugiere que estas células tienen la capacidad de transmitir señales a las fibras nerviosas circundantes (Liu *et al*., 2015; Shibukawa *et al*., 2015; Nishiyama *et al*., 2016) (40).

Kimura *et al*,. en 2016, refiere que el TRPA1 es un canal iónico polimodal que se puede activar por medio de las alquilamidas como hidróxido sanshool y componentes endógenos como: peróxido de hidrógeno, ácido nitrico-oleico, ácidos epoxieicosatrienoicos (5,6-ETT; 8,9-ETT). También los canales TRPA1 pueden ser activados por modificaciones covalentes de cisteína y lisina en el extremo N-terminal (24, 54).

Se han identificado 6 proteínas que codifican para 4 genes de ASICs los cuales son: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 y ASIC4; tienen funciones mecano-sensitivas, quimiosensitivas y nociceptivas. En donde a nivel del las neuronas trigeminales murinas se han detectado 17 de los 28 genes de canales TRP como: TRPA1, TRPC1, TRPC3,TRPC4, TRPC5, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7,TRPM8, TRPV1, TRPV2, TRPV4, TRPML1 y TRPP2; en el ganglio trigeminal humano se han identificado 10 TRP como: TRPC1, TRPM2, TRPM3, TRPM7, TRPM8, TRPV1, TRPV2, TRPV3,TRPV4 and TRPML1) y ASIC1-3. (18,19, 41).

Se sugiere que sí puede ser posible la cercanía que hay entre la empalizada odontoblástica y la porción terminal de las fibras nerviosas, cuando se estimula el odontoblasto se da la liberación de mediadores al interior de los gaps que hay entre los odontoblastos y la fibra nerviosa, seguido de la producción de una señal aferente (28).

Se han propuesto diferentes tipos de mediadores (Liu *et al*., 2012; Bond *et al.,* 2013; Lee *et al*., 2017) como la galanina, glutamato, óxido nítrico, purinas extracelulares como el Adenosin y el ATP mediante la transmisión purinérgica (29, 30). Esta transmisión purinérgica consiste en que las purinas y pirimidinas median la respuesta celular por la estimulación de los receptores específicos; en donde cada célula tiene una reserva de ATP que bajo condiciones fisiológicas es liberado por hemicanales connexin 43, pannexin-1, canales de aniones y mecanismos dependientes de Ca2+.(31).

En cuanto a los receptores purinérgicos estos se dividen en dos categorías: receptores P1 y P2 (Bond *et al*., 2013). El P1 son receptores para adenosina, acoplados a la proteína G, se subclasifican en receptores P2Y y los receptores P2 son para el ATP y se subclasifican en canales catiónicos ionotrópicos P2X. Actualmente se han identificado en mamíferos siete receptores P2X (1R-7R) y ocho receptores P2Y (P2Y1R, P2Y2R, P2Y4R, P2Y6R, P2Y11R, P2Y12R, P2Y13R y P2Y14R) (Bond *et al*., 2013; Shibukawa *et al*., 2015; Le Fur-Bonnabesse *et al*., 2017). Los P2XR se expresan tanto en las células nociceptivas del ganglio trigeminal y en la pulpa dental (Fu *et al.,* 2015; Iwamoto *et al*., 2017) (32).

Por otra parte, se detectaron fibras nerviosas positivas a receptores P2X mielinizadas y no mielinizadas a nivel del plexo *subodontoblástico* en estrecha cercanía con los odontoblastos. Además, los odontoblastos expresan diferentes subtipos de P2XR (Lee *et al.,* 2017; Shiozaki *et al*., 2017) lo que sugiere que el ATP podría regular la función fisiológica de los odontoblastos (Iwamoto *et al.,* 2017). También, los subtipos de receptores P2Y están presentes en células de pulpa (Wang *et al*., 2016), neuronas del ganglio trigeminal (Li *et al*., 2014; Kawaguchi *et al*.,

2015), así como en los odontoblastos (Sato *et al*., 2015; Wanget *et al*., 2016; Nishiyama *et al*., 2016) (41, 42).

Por los estudios recientes acerca del odontoblasto, se ha determinado que el estímulo mecánico o la estimulación directa de los odontoblastos conducen a la liberación de ATP, presumiblemente a través de canales iónicos (43). Por ejemplo, la activación del TRPA1 y TRPV4, pero no del receptor TRPV1, en células humanas similares a odontoblastos produce un incremento en la liberación de ATP la cual se puede inhibir mediante los antagonistas selectivos de TRP (Ikeda *et al.,* 2013; Khatibi *et al.,* 2015; Le Fur-Bonnabesse *et al.,* 2017) (44, 45, 53).

Por otra parte, ya se ha evidenciado que los odontoblastos constituyen una barrera importante contra la infección bacteriana (caries dental). En donde la supervivencia de estas células son fundamentales para el pronóstico de la pulpa inflamada. (Li *et al*., 2018) sugiere que la autofagia es fundamental para la respuesta antiinflamatoria por parte de los odontoblastos, teniendo en cuenta que la autofagia se conserva evolutivamente reciclando proteínas de larga longevidad junto con organelos lesionados para poder generar una homeostasis a nivel intracelular (68, 69).

Zhang *et al.* en el 2018, reportan que la progresión de la autofagia incluye la formación de vesículas de doble membrana que pueden expandirse en autofagosomas y este proceso es importante tanto en la regulación fisiológica como patológica como en el cáncer, la neurodegeneración e inflamación (70). La autofagia también participa a nivel del desarrollo dental y el envejecimiento de los odontoblastos (Couve *et al*., 2011; Couve *et al*., 2012; Takanchea *et al*., 2018 ; Li *et al*., 2018; Zhang *et al*., 2018) (71, 72, 73, 74)

Li *et al*., mencionan que los mecanismos de la regulación de la autofagia son complejos y que a su vez hay muchos factores transcripcionales como la familia FoxO, TFEB y el CLOCK los cuales regulan la autofagia en respuesta a diferentes estímulos tanto intra como extracelulares. Pero los factores de transcripción específicos responsables de activar e inducir por el proceso inflamatorio en los odontoblastos no han sido identificados hasta la actualidad (68).

En donde la familia del FoxO está conformado por: el FoxO1, FoxO3a, FoxO4 y las subfamilias FoxO6 en células de mamíferos. Sin embargo el FoxO3a ha sido destacado debido a su regulación de la inmunidad, longevidad, metabolismo, diferenciación y apoptosis celular. El FoxO3a puede ser fosforilado e inhibido por PI3K/Akt y la disminución de este factor en células tumorales promueve la proliferación, conllevando al cáncer; también se ha encontrado que tiene función pro-apoptótica, optimizando las funciones protectoras de la autofagia frente al estrés ambiental (68, 70). Los odontoblastos pueden usar FoxO3a como un sensor para detectar el estrés celular (68).

La activación de Akt puede desencadenar varias respuestas biológicas, su fosforilación reduce la activación transcripcional de FoxO3a por la inhibición de la traslocación de FoxO3a al núcleo celular. Li *et al.,* en el 2018, reportaron cuando el FoxO3 fue activado y se dio la traslocación del núcleo, se observó un aumento de los marcadores de la autofagia en estadios iniciales; sugiriendo que la autofagia podría ser provocada por FoxO3a para proteger las células en el estadío inicial. En estadios avanzados, la autofagia va disminuyendo, pero FoxO3a va aumentando y el p-Akt fue regulado positivamente, planteando que el Akt está implicado en la protección contra la muerte celular e inflamación en lugar de FoxO3a. Por lo tanto se propone que el FoxO3a tiene como función la regulación de la activación de la autofagia para la supervivencia de los odontoblastos (68).

Los canales nociceptivos de TRP participan en un múltiples modalidades de percepción del dolor como el dolor inflamatorio, dolor neuropático, dolor visceral y dolor relacionado bajo condiciones patológicas como el cáncer y migraña (60).

Por ejemplo, la participación de TRPV1 a nivel del dolor inflamatorio es el más expresado entre los canales de TRP. Sin embargo, los antagonistas de TRPV1 son eficientes disminuyendo la hiperalgesia térmica bajo condiciones inflamatorias y aumentando la percepción del dolor urente nociceptivo (61). Además, la evidencia científica respalda el papel del TRPV1 y TRPA1 en la pulpitis sintomática, sugiriendo que median la hiperalgesia tanto al frío como al estímulo mecánico (62).

Por otra parte, el óxido nítrico (NO) tiene una participación en el proceso inflamatorio el cual es sintetizado y expresado por enzimas neuronales y endoteliales (e) NO sintasas (NOS) o por la enzima inducible (i) expresada en iNOS. En respuesta frente a estímulos fisiológicos se desencadena una señal de Ca2 +a nivel intracelular a su vez activando el nNOS y eNOS (Korkmaz *et al*., 2010). Pero en las células en reposo el iNOS no se expresa, pero está regulado a nivel de los genes por mediadores inflamatorios, como lipopolisacáridos bacterianos (LPS) o citoquinas proinflamatorias como la interluquina IL-1, TNF-α, IFN-γ.

En concentraciones fisiológicamente bajas, (Korkmaz *et al.,* 2011) el NO es producido por nNOS y eNOS e interactúa directamente con el hierro y así activar la enzima para producir la molécula efectora intracelular de guanosina cíclica 3 ', 5'-monofosfato (cGMP); en donde la formación de dicha molécula cGMP a su vez desencadenará una gran variedad de respuestas celulares como: proliferación y diferenciación celular, neurotransmisión y vasodilatación (65, 66).

En cuanto a la enzima soluble de gunilato ciclasa de No (sGC), es una enzima heterodimérica que consiste en unas subunidades α y β, y la expresión de ambas subunidades se requiere para la actividad catalítica de la enzima, se han identificado la existencia de las subunidades α2 y β1 de sGC y formación dependiente de NO de cGMP en odontoblastos (Korkmaz *et al.,* 2011). También se ha encontrado que la inflamación del complejo dentino-pulpar induce una disminución a los niveles de proteína α1-, β1- y de la subunidad α2 de sGC en los odontoblastos. En donde el proceso inflamatorio se da la producción de especies reactivas de oxígeno (ROS), de especies reactivas de nitrógeno (RNS) y de los mediadores inflamatorios; los cuales pueden estar involucrados en la reducción de las subunidades α1, β1 y α2 del sGC en los odontoblastos. Por lo tanto se sugiere que el sGC es una enzima crítica para modular los diferentes efectos biológicos del NO en odontoblastos sanos (65, 72).

Song *et al*., 2017 refieren que el pannexin desempeñan un rol crucial en la modulación de la homeostasis tisular y la regulación de la patogénesis de los diferentes estados inflamatorios. Se sugiere que el Panx3 contribuye con interacción entre las células inmunes y musculares, participa en diferentes vías de señalización intracelular modulando la expresión génica y actividad celular (74). Además, Song *et al*., en el 2017 evaluaron que el Panx3 se manifiesta en el tejido pulpar y en las células odontoblásticas humanas, planteando una hipótesis de que únicamente el Panx3 desempeña un papel fundamental durante el tratamiento de la inflamación pulpar, pero no hay estudios que confirmen esta hipótesis actualmente. He informó que el Panx3 regula la expresión de citoquinas proinflamatorias como IL-1b e IL-6 en respuesta a la estimulación de TNF-α mediante la vía de señalización NF-κB. (77, 78).

*Odontoblastos y respuesta Inmune:*

Para que se de una respuesta inmunulógica competente y adecuada frente a la invasión microbiana, se debe de tener una célula especializada la cual detecte rápidamente los patógenos con unos receptores tanto extracelulares como intracelulares.

Los NLR y TLR tienen como función el reconocimiento de una gran variedad de molécuas asociadas a patógenos (PAMPs) (79). Los TLR son unos receptores los que están anclados a nivel de la membrana celular, actúan a nivel extracelular, representan la primera línea de defensa y hasta la actualidad se han descrito diez TLR humanos por ejemplo, y el TLR4 reconoce los componentes de las bacterias Gram-negativas como el lipopolisacárido (LPS) (80). Por otro lado, los NLR actúan a nivel intracelular y representan una segunda línea de defensa contra los patógenos que evaden la superficie celular (81).

Las células tipo odontoblasto expresan genes TLR1, TLR6, TLR9 (Farges *et al*., 2009; Staquet *et al*., 2011; Jang *et al*., 2015).; sugiriendo que el odontoblasto tiene la capacidad de reconocer una variedad de PAMPs como: lipopéptidos diacetilados (TLR2, TLR6), RNA viral (TLR3), Flagelina (TLR5), LPS (TLR4), lipopéptidos triacetilados (TLR1,TLR2) (82, 83).

Según Yumoto *et al*., 2018, por medio estudios con microarray de DNA y PCR reporta que el TLR3, TLR7, TLR8 se han detectado en odontoblastos y en el tejido pulpar. En donde el TLR8 incrementa su expresión en los odontoblastos; sugiriendo que este puede reconocer RNA viral participando así de la respuesta inmune innata a nivel del complejo dentino-pulpar (84, 85).

Los odontoblastos expresan la familia de proteínas NRL como el NOD1 el cual lo expresan los tejidos pulpares humanos frente a un proceso infeccioso o sin este, a nivel del citoplasma de los odontoblastos y de células del endotelio vascular, pero si hay un proceso infeccioso en curso se expreso el NOD1 en la capa odontoblástica radicular (86, 87).
También expresan los odontoblastos unos péptidos las cuales son las beta-defensinas (hBD), cuya función es la disrupción de la integridad de la membrana plasmática del microorganismo. Se ha evidenciado la expresion de hBD-1 y hBD-4 en los dientes sanos (88, 89). El hBD-1 se expresa en niveles minimos en el citoplasma del odontoblasto y el hBD-2 es indetectable, pero si hay una detección del LPS a nivel de estudios se sugiere que se expresa el hBD-2 (90, 91).

*RESULTADOS*

La búsqueda en la base de datos electrónica, actualizada por última vez el 25 de Junio de 2018 arrojó 492.157 publicaciones, de las cuales 0 se encontraron en MEDLINE, 160 en EMBASE, 147 en Science Direct y 27 en Lilacs. Después de la evaluación de los títulos y los resúmenes se eliminaron inmediatamente 521 artículos. Los 150 artículos restantes fueron examinados y excluidos si no cumplían con los criterios de inclusión o se encontraban duplicados dando como resultado un total de 94 artículos aceptados para la revisión final y procesados para la extracción de datos.

DISCUSIÓN

Brannström *et al*., en 1966 refieren que la sensibilidad dentinal se explicaba generalmente por la teoría hidrodinámica, que afirma que los estímulos externos inducen movimientos del fluido dentinal que activan a su vez las fibras nerviosas en los túbulos dentinarios y producen un dolor transitorio (2).

Pero Ajcharanukul *et al*., en 2011 cuestino esta hipótesis ya que se han realizado observaciones recientes, reportando que los movimientos del fluído en los túbulos dentinarios no están directamente asociados con la sensibilidad dentinal y el dolor dental (20). Tambien Chung *et al*., en 2014, sugieren que los canales iónicos que expresa el odontoblasto están involucrados en la transducción de estímulos nocivos cuando el estímulo se traduce en señales eléctricas en los aferentes nociceptivos de la pulpa dental, dicha información es interpretada y procesado por áreas corticales superiores, lo que resulta en la experiencia dolorosa. Por lo tanto si se aplican estímulos térmicos al diente evoca dolor al activar los nervios que inervan la pulpa dental (31).

Jardín *et al*., en 2017 reportan que el TRPA1 es un canal iónico polimodal que puede ser activado por estímulos físicos y químicos. Entre los estímulos físicos el TRPA1 es sensible a la temperatura. En donde 10 canales TRP termoeléctricoss han sido identificados hasta la fecha como TRPV1-4, TRPM2, TRPM4, TRPM5, TRPM8, TRPC5 y TRPA. Así mismo, TRPV4 activado por temperaturas entre 27 y 42 °C, el TRPV2 es activado por el calor >52 ° C, el TRPV1 es sensible a >42 °C, el TRPV3 >33 ° C, el TRPM2 sensible a temperaturas entre 35 y 42 °C, el TRPM4 y TRPM5 sensible a las temperaturas entre 15 y 35 °C. Sin embargo, la actividad TRPC5 es aumentada a temperaturas por < 30 °C, TRPM8 es sensible a las temperaturas por debajo de 25 ° C y TRPA1 se activan a temperaturas <17 °C (Vriens *et al*., 2014). En donde el TRPV1 se ha relacionada con sensación urente, se ha informado que el TRPA1 está asociado a la nocicepción del frío (40).

Chen *et al*., 2013 reporta que en mamíferos, los canales TRP se han clasificado en 6 subfamilias sobre la base de homología de secuencia de aminoácido. Masuda *et al*., en 2017 mencionan que en modelo animal, los canales de TRP han sido relacionados a la producción del dolor facial inflamatorio y neuropático; suguiriendo que estos canales están involucrados en la transducción de estímulos nocivos, cuando el estímulo se transduce en señales eléctricas a nivel de los receptores nociceptivos de la pulpa dental; la información es luego interpretada

y procesado por áreas corticales superiores, lo que resulta en la experiencia de dolor (30). Aplicando estímulos térmicos al diente conlleva al dolor al activar los nervios que inervan la pulpa dental. En condiciones inflamatorias que involucran pulpa dental, esta se presenta hipersensibilidad y puede conducir a cambios térmicos, mecánicos e hipersensibilidad osmótica, es decir, sensibilidad exagerada al frío, calor, estímulos mecánicos y osmóticos en el diente. Actualmente los mecanismos moleculares implicados en la hipersensibilidad siguen sin esclarecerse (39).

El Karim *et al*., 2011 reporta que la superfamilia de TRPs está conformada por seis miembros TRPA1, TRPM8, TRPV1, TRPV2, TRPV3 y TRPV4, proponiendo que estos participan en la termo-sensibilidad y a su vez se detectaron en los nervios sensoriales, pero solo algunos de ellos de ellos se han detectado en los odontoblastos y en las neuronas aferentes del trigémino (Byers *et al*., en 2012). El TRPV1, TRPV2, TRPV3 y TRPV4 tienen funciones de percepción desde el frío hasta el calor. Mientras que TRPA1 y TRPM8 responden al frío, el TRPV1 y TRPV2 se activan mediante niveles dolorosos de calor (> 43 ° C y > 52 ° C), TRPV3 y TRPV4 responden a calor no doloroso (33-39 ° C), el TRPM 8 se activa con temperaturas frías no dolorosas (<25 ° C) y el TRPA 1 se activa con un frío doloroso (<18 ° C) (El Karim *et al*., 2011; Kim *et al*., 2012; Sato *et al*., 2013)TRPV1 también responde a estímulos nocivos y diversos agentes químicos (22, 44, 50).

El TRPV1, TRPV2 y TRPA1 también se expresan en pacientes con pulpitis (Story *et al*., 2003; Park *et al*., 2006; Chung *et al*., 2013). En la pulpa dental humana, TRPA1 se expresó en una gran cantidad de axones que se ramifican en la pulpa periférica así como en el cuerpo celular de los odontoblastos (Kim *et al*., 2012) (20, 36, 50).

Además, muchos canales de la membrana plasmática tipo neuronal han sido hallados en odontoblastos, sugiriendo que pueden crear potenciales de acción en situaciones específicas. Sin embargo, el voltaje los canales iónicos en los odontoblastos pueden permitir una actividad similar a la glía23 para ayudar a las terminaciones nerviosas sensoriales que se extienden más allá de la Glía terminal en la pulpa (40, 48).

Según Tsumura *et al* en 2012, demostraron que el aumento de [Ca2 +] i inducido por 2-AG era sensible al antagonista del receptor CB1 AM251, lo que indica que los odontoblastos expresan receptores CB1 pero no CB2. Los receptores CB1 tiene funciones para el efecto antihiperalgésico y antinociceptivo en el dolor (15).

Ichikawa *et al*., en 2012, Iindican que los canales depedientes de voltaje de Na+, no tiene un rol importante en condiciones fisiológicas en los odontoblastos, pero estos pueden ser activados cuando hay una interacción entre BK que induce a la movilización del Ca+ intracelular y que a su vez se abran los canales de Ca2+ y así se produzca el potencial de membrana. Sugiriendo que los canales de Na+ en odontoblastos humanos tienen un papel importante en dirigir las funciones celulares como la secreción de dentina y la nocicepción durante la inflamación pulpar por el aumento en la apertura de los canales de Na+ (14, 20, 24).

Los odontoblastos son la primera barrera encontrada por la invasión de patógenos en la dentina y la supervivencia de los odontoblastos está directamente relacionada con su autofagia en un microambiente inflamatorio; en donde en una etapa inicial la autofagia inducida por LPS, teniendo los odontoblastos este mecanismo de pro-supervivencia. Así mimos, en una etapa avanzada si la estimulación es constante por LPS este va a inducir tanto a la autofagia como a la apoptosis celular.

El FoxO3a tiene un rol importante en la autofagia, el cual tiene un papel pro-supervivencia en lugar de apoptosis. Se sugiere que el FoxO3 participa en la autofagia, para que se mantenga la viabilidad y la homeostasis celular bajo condiciones fisiológicas, pero una infección leve activa la autofagia para mejorar la supervivencia celular bajo estrés (70, 72).

Por otro lado, se ha descrito que la respuesta de la pulpa al proceso infeccioso y la lesión es similar a la de muchos otros tejidos en el cuerpo humano, las células del complejo dentino-pulpar son capaces de detectar bacterias invasoras por su expresión de PRR, los cuales

identifican PAMPs (Tsumura M *et al*., 2013; Sato M, *et al*., 2015) (80, 86, 88). Los PRR reportados presentes en la pulpa son los Toll-1 y Toll-2, el receptor similar al Nod3 conocido como el inflamasoma.

A su vez la expresión de varias de estas moléculas se ha identificado en céulas endoteliales, odontoblastos, neuronas, fibroblastos de pulpa, células madre de la pulpa, teniendo la capacidad de sensar componentes extracelulares (LPS) e intracelulares (ADN) de las microorganismos que migran hacia los túbulos dentinales (92, 93, 94).

## FUTURAS INVESTIGACIONES

A partir de las publicaciones recientes y con los hallazgos de este estudio, se ha evidenciado múltiples funciones del odontoblasto a nivel inmunológico, vascular y neuronal como modulador de las respuestas celulares y moleculares en el complejo pulpo-dentinal, se puede centrar diversas terapéuticas clínicas que pueden favorecer su potencial rol en los procesos regenerativos y/o reparativos en el complejo pulpo-dentinal.

## CONCLUSIONES

Este estudio examinó y comparó la litertura disponible actualmentes acerca de las múltiples funciones del odontoblasto como la nocicepción, la respuesta sensorial a múltiples estímulos externos, la capacidad inmunológica y la partipación en procesos inflamatorios, encontrando que esta célula especializada tiene la capacidad de participar y modular los procesos fisiopatológicos tanto de la dentina como de la pulpa, ampliando las perspectivas a las terapeúticas actuales favoreciendo los procesos de regeneración y/o reparación en el complejo pulpo-dentinal hacia una mayor investigación en el área para poder determinar cómo alcanzar dichas terapéuticas clínicas.

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