

The role of β -catenin in tooth development

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الخلاصة

هذه الدراسة أجريت على مراحل تكوين الاسنان خلال المرحلة الجنينية لحيوان الفار، والهدف من اجراء هذه الدراسة هو لبيان مدى تعبير وفعالية البيتا- كاتنين (β -Catenin) في عملية تكوين الاسنان وتشكيلها. هذه الدراسة شملت (21) مقطع تم اختيارها من مراحل تكوين الاسنان المختلفة (مرحلة البرعم والقبة والجرس) ، وتم اختيار (7) مقاطع من كل مرحلة التعبير عن (β -Catenin) تم باستعمال الصبغة الكيميائية النسجية المناعية لهذا البروتين، وتمت الصبغة على مقاطع بسمك (4) مايكرومتر. اظهرت النتائج وجود صبغة نوية وسائتوبلازمية شديدة في الخلايا المينية الداخلية والخلايا المينية الخارجية والخلايا الجاورة لهما وهي الخلايا اللبية السنية والخلايا التي تحيط ببرعم السن، بينما الخلايا النجمية الشبكية تظهر صبغة سائتوبلازمية متوسطة الشدة وعدم وجود صبغة نوية، ان البيتا كاتنين يظهر تعبيراً بنسبة عالية خلال مراحل تكوين الاسنان وهذا يعود الى دوره في انتقال الاشارة الخلوية بين عقدة المينا والخلايا المحيطة، للتحفيز على تكوين الاسنان وتشكيلها.

Abstract

Background:The present experimental study was conducted on tooth development during embryogenesis in developing mouse.

with main objective to identify the expression and action β -Catenin in

development of teeth and morphogenesis.

Materials and Methods:This study comprised a total of (21) sections were selected during different stages of tooth development (bud, cap and bell stage), (7) sections from each stage in developing mouse.

The expression of Beta-Catenin was carried out on 4 μ m specimen sections using Immuno histochemical staining of β -Catenin antibody.

The staining demonstrated the expression intensity.

Results:Results shows intense nuclear and cytoplasmic staining in inner enamel epithelium, outer enamel epithelium and in adjacent dental papilla and ecto-mesenchymal cells surrounding the tooth germ, that will give rise to a definite dental sac,

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while the stellate reticulum, shows only moderate cytoplasmic and not nuclear staining.

Conclusion: β -catenin is highly expressed during tooth development stages due to its role in cell signaling transmission between the enamel knot and the surrounding mesenchymal cells to induce tooth formation and its morphogenesis.

Introduction

Beta-Catenin (β -Catenin) is a multifunctional protein playing an essential role in cell-cell adhesion by binding to the transmembrane protein, cadherin.

β -Catenin is also involved in the regulation of the Wnt signaling pathway.

The Wnt signaling pathway describes a network of proteins most well known for their roles in embryogenesis and cancer, but also involved in normal physiological processes in adult animals (1).

Tissue and organ development of vertebrates is tightly regulated by signaling cascades that act in a spatially and temporarily controlled manner.

Prominent examples of these tightly controlled signaling pathways include the canonical Wnt/ β -catenin and the TGF β /Nodal signaling pathways, both of which play multiple essential roles in early embryogenesis (2).

The expression of Wnt target genes is regulated by nuclear β -catenin that is bound to transcription factors of the Lef/Tcf family (2).

Signaling by the Wnt family of secreted glycolipoproteins via the transcriptional coactivator β -catenin controls embryonic development and adult homeostasis (3).

Signaling by the Wnt family of secreted glycolipoproteins is one of the fundamental mechanisms that direct cell proliferation, cell polarity, and cell fate determination during embryonic development and tissue homeostasis (4). As a result, mutations in the Wnt pathway are often linked to human birth defects, cancer, and other diseases (5).

Wnt signaling is involved in virtually every aspect of embryonic development and also controls homeostatic self-renewal in a number of adult tissues.

Wnt/beta-catenin signaling plays key roles in tooth development, but how this pathway intersects with the complex interplay of signaling factors regulating dental morphogenesis has been unclear (6).

The Wnt/beta-catenin signaling pathway is one of several key conserved intercellular signaling pathways in animals, and plays fundamental roles in the proliferation, regeneration, differentiation, and function of many cell and tissue types. This pathway is activated in a dynamic manner during the morphogenesis of oral organs, including teeth, taste papillae, and taste buds, and is essential for these processes to occur normally. Conversely, forced activation of Wnt/beta-catenin signaling promotes the formation of ectopic teeth and taste papillae (7).

Mutation of beta-catenin to a constitutively active form in oral epithelium causes formation of large, misshapen tooth buds and ectopic teeth, and expanded expression of signaling molecules important for tooth development. Conversely, expression of key morphogenetic regulators including Bmp4, Msx1, and Msx2 is down regulated in embryos expressing the secreted Wnt inhibitor Dkk1 which blocks signaling in epithelial and underlying mesenchymal cells. Similar phenotypes are observed in embryos lacking epithelial beta-catenin, demonstrating a requirement for Wnt signaling within the epithelium. Inducible Dkk1 expression after the bud stage causes formation of blunted molar cusps, down regulation of the enamel knot marker p21, and loss of restricted ectodin expression, revealing requirements for Wnt activity in maintaining secondary enamel knots.

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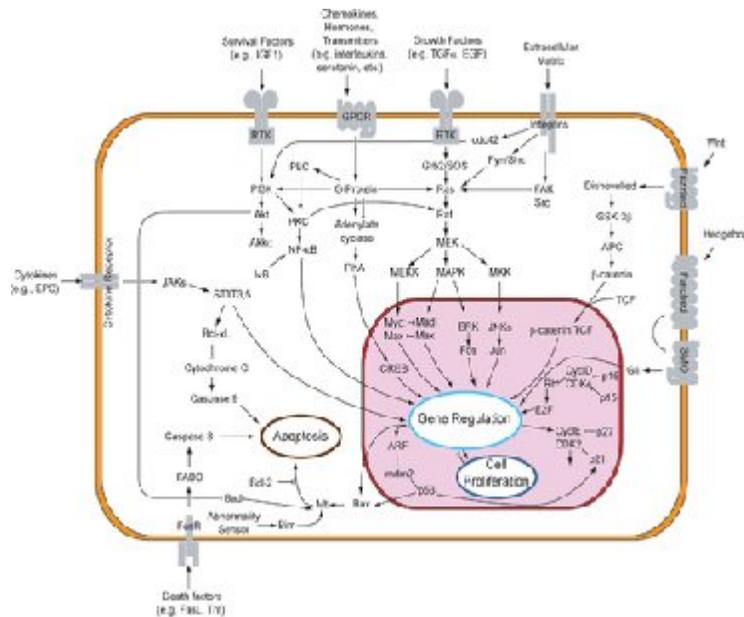


Figure.1: Overview of signal transduction pathways. On the upper right hand side of the cell, a Wnt signaling protein is shown to bind to a frizzled receptor.

Objective

Identification of B-catenin expression and its action during tooth development.

Materials and Methods

-Selection of cases and tissue staining:

An experimental study was carried out at the Surgical Pathology Center in Davanzo Hospital-Foggia-Italy, between April 2008 - July 2008, involving 21 sections (4 μ m) from formalin – fixed paraffin embedded blocks were cut during different stages of tooth development (bud, cap and bell stage), (7) sections from each stage of developing mouse.

Immuno histochemistry staining was then performed on the sections that mounted on poly- L- lysine coated glass slides utilizing mouse monoclonal β -catenin antibody (8).

After antigen retrieval by pressure cooking with ethylene diamine tetra-acetic acid (EDTA) solution as a buffer solution, and quenching in 30% hydrogen peroxide and blocking. The sections were incubated with primary antibody B-catenin. Then biotinylated anti rabbit immunoglobulin and streptavidin conjugated to horse radish peroxidase (HRP) were subsequently applied. Finally , 3,3-diaminobenzidine was used for color development and haematoxylin was used for counter staining.

The results of Immuno histochemical staining were evaluated by two observers which appear as brown pigmentation.

Results

Staining results show

-Intense nuclear B-Catenin expression in inner enamel epithelium and its adjacent dental papilla (fig. 2 & 4) in whole examined sections with (+++) intensity in comparison with the surrounding tissue cells.

- also we found intense nuclear staining (+++) in convex outer enamel epithelium and the ecto-mesenchymal cells surrounding the tooth germ, that will give rise to a definite dental sac in the

following stage of differentiation, showed strong nuclear staining (fig. 2).

- The inner enamel epithelium and outer enamel epithelium closely adjacent to the addensed mesenchyme shows intense nuclear and cytoplasmic positivity for beta-catenin, while the stellate reticulum, not directly influenced by the mesenchyme showed only moderate cytoplasmic and not nuclear staining (fig. 3).

- B-catenin shows membranous expression in the dental lamina (fig.5).

-Cytoplasmic expression of beta-catenin were seen in the basal layer of epithelium lining the primitive oral cavity in a region near a developing tooth bud. The upper spinous layer show intense membranous and faint cytoplasmic staining. No nuclear staining in the upper layer of epithelium is appreciable, while scanty nuclear-beta catenin positive cells are evident in the cells under the basal lamina (fig. 6).

Figure (7 & 8) show bud and cap stages of tooth development with hematoxylin stain.

Figure (9) shows the localization of enamel knot.

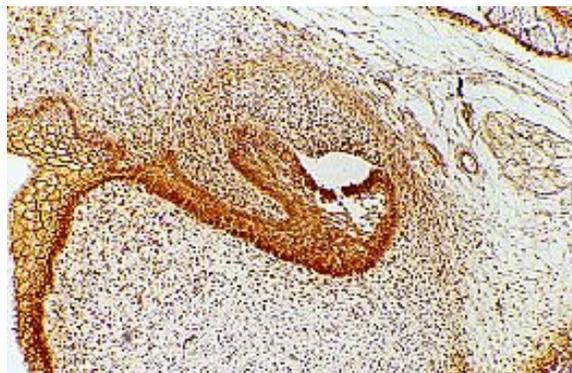


Figure.2: Beta-catenin expression in early bell stage of developing mandibular tooth. Note the intense nuclear staining for beta catenin in the inner epithelium adjacent to the dental papilla, which also express intense nuclear-beta-catenin. Also the outer convex epithelium and the ecto-mesenchymal cells surrounding the tooth germ, showed strong nuclear staining. LSAB-HRP, nuclear counterstaining with hematoxyllin.

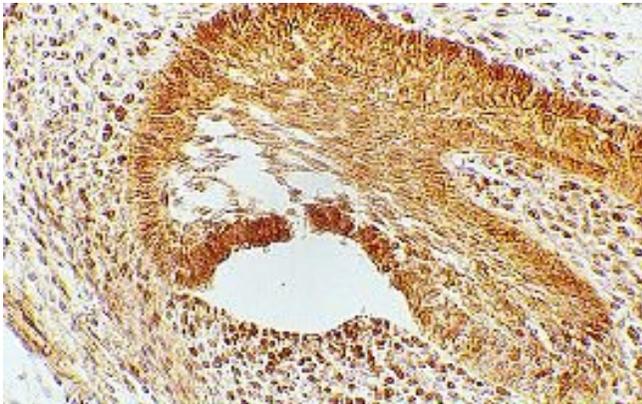


Figure. 3: Expression of beta catenin in early bell stage. Note the intense and high percentage of nuclear staining in addensed mesenchyme of dental papilla and in mesenchyme surrounding the enamel organ. Note that the inner enamel epithelium and outer enamel epithelium closely adjacent to the addensed mesenchyme shows intense nuclear and cytoplasmic positivity for beta catenin, while the stellate reticulum, not directly influenced by the mesenchyme showed only moderate cytoplasmic and not nuclear staining.

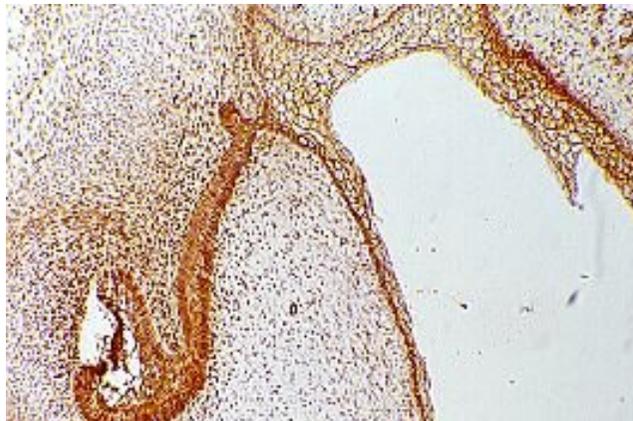


Figure. 4: Beta catenin expression in a developing mandibular tooth germ. Note the maximum expression of nuclear staining in the inner enamel epithelium and in the addensed mesenchyme of dental papilla.LSAB-HRP.



Figure. 5: Beta catenin membranous expression in the dental lamina (dental ridge) near a developing maxillary tooth. Note the placode at the origin of dental bud that probably represents a future successional bud that will give rise from the gubernacular cord of the deciduous tooth, expressing cytoplasmic staining for beta catenin.



Figure.6: High magnification field showing cytoplasmic expression of beta-catenin in the basal layer of epithelium lining the primitive oral cavity in a region near a developing tooth bud. The upper spinous layer show intense membranous and faint cytoplasmic staining. No nuclear staining in the upper layer of epithelium is appreciable, while scanty nuclear-beta catenin positive cells are evident in the cells under the basal lamina. LSAB-HRP technique.

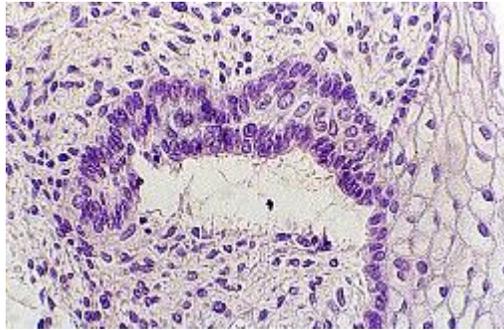


Figure.7: Early dental bud. Hematoxyllin stain.

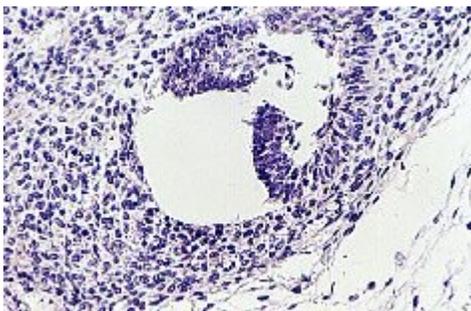


Figure. 8: Cap stage. Hematoxyllin stain.

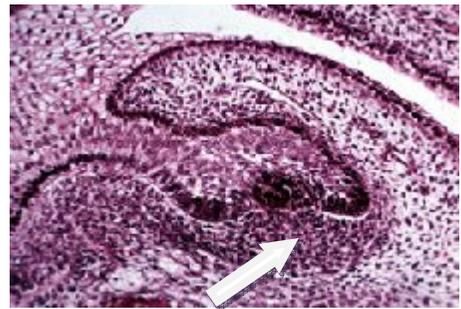


Figure.9: Cap stage. The arrow shows enamel knot. H&E stain.

Discussion

Odontoblasts differentiate from the cells of the dental papilla, and it has been well-established that their differentiation in developing teeth is induced by the dental epithelium. In experimental studies, no other mesenchymal cells have been shown to have the capacity to differentiate into odontoblasts, indicating that the dental papilla cells have been committed to odontoblast cell lineage during earlier developmental stages. The advancing differentiation within the odontoblast cell lineage is regulated by sequential epithelial signals. The first epithelial signals from the early oral ectoderm induce the odontogenic potential in the cranial neural crest cells (9). The next step in the determination of the odontogenic cell lineage is the development of the dental papilla from odontogenic mesenchyme. The formation of the dental papilla starts at the onset of the transition from the bud to the cap stage of tooth morphogenesis, and this is regulated by epithelial signals from the primary enamel knot. The primary enamel knot, is a signaling center which forms at the

tip of the epithelial tooth bud. It becomes fully developed and morphologically discernible in the cap-stage dental epithelium and expresses at least ten different signaling molecules belonging to the BMP, FGF, Hh, and Wnt families. In molar teeth, secondary enamel knots appear in the enamel epithelium at the sites of the future cusps (9) They also express several signaling molecules, and their formation precedes the folding and growth of the epithelium. The differentiation of odontoblasts always starts from the tips of the cusps, and therefore, it is conceivable that some of the signals expressed in the enamel knots may act as inducers of odontoblast differentiation. The functions of the different signals in enamel knots are not precisely known. They have shown that FGFs stimulate the proliferation of mesenchymal as well as epithelial cells, and they may also regulate the growth of the cusps. They have proposed that the enamel knot signals also have important roles, together with mesenchymal signals, in regulating the patterning of the cusps and hence the shape of the tooth crown. They suggest that the enamel knots are central regulators of tooth development, since they link

Cell differentiation to morphogenesis (9).

β -catenin stabilization results in its higher nuclear levels (10).

Earlier studies suggested that β -catenin enters the nucleus in a nuclear localization signal (NLS)- and importin-independent fashion by interacting directly with nuclear pore proteins (10), Axin (11), and Ran binding protein 3 (RanBP3), which binds to β -catenin in a Ran- GTP-dependent manner (12).

Our study shows intense nuclear and cytoplasmic B-catenin expression in both epithelial (inner enamel epithelium, outer enamel epithelium) and mesenchymal (dental papilla, dental sac) tissues, which are responsible for cell signaling and then cell differentiation and morphogenesis during tooth development. These findings were in agreement with the other studies mentioned in this study, explaining the role of B-Catenin in tooth development and morphogenesis.

Conclusion

β -catenin is highly expressed during tooth development stages due to its role in cell signaling transmission between the enamel knot and the surrounding mesenchymal cells to induce tooth formation and its morphogenesis.

References

- 1- Lie DC, Colamarino SA, Song HJ, Désiré L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 437 (7063): 1370-5. doi:10.1038/nature 04108. PMID 1625, 1967.
- 2- Arnold SJ, Stappert J, Bauer A, Kispert A, Herrmann BG, and Kemler R(2000). Brachyury is a target gene of the Wnt/beta-catenin signaling pathway. *Mech. Dev.* 91, 249 -258.
- 3- Bryan TM, Keiko T, Xi H (2009). Wnt/B-Catenin Signaling: Components, Mechanisms, and Diseases. *Developmental cell*, 17, 1, 9-26.
- 4- Logan CY, and Nusse R (2004). The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.*, 20, 781- 810.
- 5- Clevers H (2006). Wnt/B-Catenin signaling in development and disease. *Cell*, 127, 469- 480.
- 6- Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, Yang SH, Lu MM, Piccolo S, Ullrich R, Taketo MM, Morrissey EE, Atit R, Dlugosz AA, Millar SE, (2008). Wnt/Beta-Catenin signaling directs multiple stages of tooth morphogenesis. *Dev. Biol.*, 1, 313(1), 210-224.
- 7- Liu F, Millar SE, (2010). Wnt/B-Catenin signaling in oral tissue development and disease. *J Dent Res.*, 89(4), 318-330.
- 8- Harboe N, (2005). B-Catenin monoclonal mouse anti-human. *Immunocytochemistry*. 113.
- 9- Thesleff I, Keranen S, Jernevall J, (2001). Enamel knots as signaling centers linking tooth morphogenesis and odontoblast differentiation. *Adv Dent Res.* 15, 8-14.
- 10- Henderson BR, and Fagotto F, (2002). The ins and outs APC and b-Catenin nuclear transport. *EMBO Rep.*, 3, 834- 839.

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- 11-** Cong F, and Varmus H, (2004). Nuclear- cytoplasmic shuttling of Axin regulates subcellular localization of B-Catenin. *Proc. Natl. Acad. Sci. USA* 101, 2882 – 2887.
- 12-** Hendriksen J, Fagotto F, Van der Velde H, Van schie M, Noordermeer J, Fornerod M, (2005). RanBP3 enhances nuclear export of active Beta-Catenin independently of CRM1. *J. Cell Biol.*, 171, 785- 797.