INVITED REVIEW

β catenin in health: A review

Sharada Prakash, Uma Swaminathan

Department of Oral and Maxillofacial Pathology, AECS Maaruti College of Dental Sciences, Bengaluru, Karnataka, India

Address for correspondence:
Dr. Sharada Prakash,
Department of Oral and Maxillofacial Pathology,
AECS Maaruti College of Dental Sciences,
Bengaluru - 560 076, Karnataka, India.
E-mail: dkoppol@yahoo.com

Received: 17-08-2015
Accepted: 21-08-2015

ABSTRACT

β catenin belongs to the armadillo family of proteins. It plays a crucial role in developmental and homeostatic processes. Wnts are a family of 19 secreted glycoproteins that transduce multiple signaling cascades, including the canonical Wnt/β catenin pathway, Wnt/Ca^{2+} pathway and the Wnt/polarity pathway. This is a review on β catenin, Wnt proteins and their secretion, the signaling pathway, the associated factors and the crucial role of β catenin in odontogenesis.

Key words: Odontogenesis, Wnt proteins, Wnt signaling, β catenin

INTRODUCTION

β catenin belongs to the armadillo family of proteins.[1,2] Armadillo is the Drosophila homolog of vertebrate β catenin.[3] It is a multitasking and evolutionary conserved molecule and exerts a crucial role in a multitude of developmental and homeostatic processes.[1,2] It is found at multiple subcellular localizations, including junctions where it contributes to the stabilization of cell-cell contacts, cytoplasm where β catenin levels are controlled by protein stability regulating processes and nucleus where β catenin is involved in transcriptional regulation and chromatic interactions.[3] The word catenin is derived from "Catena" "Chain" in Latin.[4]

The catenin family is composed of three subfamilies, the P120 subfamily, the β subfamily (β catenin and plakoglobin) and α subfamily.[4]

The P120 subfamily contains 9 arm repeats, β subfamily contains 12 arm repeats, but the α subfamily contains three vinculin homolog domains instead of arm repeats and belongs to the Vinculin superfamily. Thus, Zhao *et al*. suggested that catenins should not be called a family, it is just a group of proteins binding C-terminal of classical cadherins.[4]

HISTORY

In the late 1980s, β catenin was independently discovered twice on the basis of its different functions: Structural and signaling. The group of Rolf Kelmer isolated β catenin together with α catenin and γ catenin, as proteins associated with E-cadherin, the key molecule of Ca^{2+} dependent cell adhesion. These proteins were named Catenins to reflect their linking of E-cadherin to cytoskeletal structures.[1]

The signaling potential was exposed through its Drosophila ortholog armadillo. Experiments on armadillo performed by the laboratories of Eric Wieschaus and Mark Peifer revealed the conservation of its structural function in Adherens junctions. Epistatic analysis revealed that armadillo segmentation function is regulated by Wingless (Wg). This was the key step in the characterization of Wnt/β catenin signaling cascade, and of the functions of its individual components. Another important discovery was the description of the basic pathway leading from Wg ligand through disheveled (Dsh) to glycogen synthase kinase-3 (GSK3).[1]

In mid-1990s, several groups found independently that the signaling function of β catenin in the nucleus is mediated via T-cell factor (Tcf) or Lymphoid enhancer-binding factor (Lef) transcription factors.[1]

STRUCTURE

β catenin consists of 130 amino acid amino-terminal domain, 12 imperfect repeats of 42 amino acids termed arm repeats and

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.164537

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Prakash S, Swaminathan U. β catenin in health: A review. J Oral Maxillofac Pathol 2015;19:230-8.

© 2015 Journal of Oral and Maxillofacial Pathology | Published by Wolters Kluwer - Medknow

carboxy-terminal domain (CTD) of 100 amino acids. Central domain is the most conserved. Each armadillo repeat forms three helices arranged in a triangular shape. The 12 repeats form a superhelix that features a long positively charged groove, in which the third helix of each repeat forms the floor of the groove.[5] The groove within the central domain can be divided into several sections. Armadillo repeats 5–9 form the core binding sites for Tcf and cadherin and an essential part of the binding site for adenomatous polyposis coli (APC). Armadillo repeats 3–4 form a binding site for Tcf, APC and Axin.[5] Tcf family members interact with β catenin armadillo repeats 3–10 and anchor β catenin to specific promoters of transcription.[6]

The first armadillo repeat (RI) and the armadillo repeat 11 to C terminus have been identified as essential regions for transactivation of Wnt target genes.[6]

N and C terminal domains are negatively charged, are sensitive to trypsin and thus structurally flexible.[5] Both N and C terminal tails act as "intramolecular chaperones" of armadillo repeat domains that increase binding specificity and prevent self-aggregation of armadillo repeat domain region.[6]

N terminal domain (NTD) harbors the binding site for α catenin, GSK3 and CSK-1 phosphorylation sites that are recognized by β-transducin repeats containing proteins (β-TrCP) ubiquitin ligase.[5]

Xing *et al.* in their study on the crystalline structure of β catenin observed an α-helix (Helix–C) at the beginning of β catenin C terminal domain. They observed that Helix–C runs parallel to helix 3 of armadillo repeat 12 and that Helix–C caps the hydrophobic surface formed by C terminal end of armadillo repeat.[6]

Helix–C is presumed to play an important role in recruitment of transcriptional coactivators and in subsequent activation of Wnt-responsive genes.[6]

Wnts

Wnt proteins are a family of signaling molecules that participate in multiple developmental events during embryogenesis and tissue homeostasis, but when dysregulated lead to multiple disorders.[7] At the cellular level, Wnts regulate stem cell self-renewal apoptosis and cell motility.[8] The human genome harbors 19 Wnt genes divided into 12 conserved Wnt subfamilies.[9]

HISTORY

The first Wnt gene was isolated in 1982, then called int-1.[8] Drosophila Wg which controls segment polarity during larval development was shown to be a fly homolog of Wnt.[10] In 1989, McMahan and Moon observed a duplication of the body axis in Xenopus following injection of mouse Wnt1 mRNA into ventral blastomeres of embryo at 4-cell stage. Axis duplication was also induced by Dsh, β catenin and GSK3.[10] These observations in Drosophila and Xenopus delineated a highly conserved signaling pathway, activated by secreted Wnt proteins.[10]

- The early analysis of overexpression of Wnt1 revealed important properties of Wnts, including secretion from cells, glycosylation and association with cell surface and extracellular matrix (ECM)[8]
- The true first amino acid of a Wnt protein was identified by amino-terminal sequencing of a purified Wnt protein[8]
- Murine Wnt3A was the first purified and biochemically characterized Wnt protein due to its efficient secretion[11]
- A high-resolution structure of a Wnt protein was accomplished in Chris Garcia's laboratory.[8]

STRUCTURE

Wnt proteins are ~40 kDa in size.[9] Wnts are cysteine rich proteins of approximately 350–400 amino acids.[11] The defining property of Wnt protein is the invariant positioning of 22 cysteine residues.[8] A high-resolution crystal structure of 3.25 A° was determined for Xenopus Wnt8 in complex with cysteine rich domain of mouse Frizzled 8. This structure revealed an unusual two domain structure with amino NTD and CTD forming a protein fold. The NTD is composed of a cluster of α helices with 10 of conserved cysteine residues forming five disulfide bridges. The CTD exhibits two β sheets, maintained by six disulfide bridges.[8] Wnts are relatively insoluble and hydrophobic.[10] The insoluble nature has been explained by the discovery that these proteins are palmitoylated.[7] Treating Wnt with enzyme acyl protein thioesterase results in a form that is not hydrophobic.[7]

SECRETION

After translation and reaching the endoplasmic reticulum (ER), Wnt proteins associate with multiple processing enzymes that chaperone them on their way to the extracellular space.[8]

Murine WNT3A was the first purified and biochemically characterized Wnt protein.[11] Wg is a Wnt molecule most investigated *in vivo*.[11] Two important posttranslational modifications occur. N-linked glycosylation that is required for WNT3A secretion and lipid modification. Two types of lipid modification account for the hydrophobicity and poor solubility of Wnt proteins. The first lipidation is the addition of palmitate to cysteine 77. The second lipidation is a palmitoyl attached to serine 209. Wnt is glycosylated and lipid-modified by a porcupine in the ER. Porcupine encodes a multipass transmembrane ER protein that contains an O-acyltransferase domain suggesting a role in Wg lipid modification. Porcupine deficiency results in Wg and Wnt accumulation in the ER and diminished palmitoleoylation

at serine 209 suggesting that porcupine is responsible for lipidation.[11] Glycosylation plays an important role in control over Wnt folding and subsequent secretion. Acylation is important for Wnt activity. Komekado *et al*. 2007 reported that nonglycosylated Wnt is not acylated and subsequently not secreted.[8] Mutagenesis of serine lipid modification sites yielded nonfunctional and poorly secreted protein. In contrast, Wg carrying a mutation of acyl-modified serine was secreted and showed poor signaling activity.[8]

After posttranslational modification, Wnts are escorted by wntless (WlS) from Golgi apparatus to the plasma membrane for secretion. WlS is a transmembrane protein that localizes to Golgi apparatus, endocytic compartments and plasma membrane and is essential for secretion. The retromer complex consisting of five subunits mediates membrane protein trafficking between endosomes and Golgi apparatus. This retromer complex is required for retrieval or recycling of WlS from endosome to Golgi apparatus, mediated by direct interaction between WlS and retromer vacuolar protein sorting-associated protein (VPS35) subunit. Loss of retromer function leads to degradation of WlS in the lysosomes and reduction in Wnt secretion. Once Wnt is released from the cell, WlS is recycled by endocytosis and trafficked back to Golgi by retromer.[11] WlS is cleared from plasma membrane by endocytosis in a process that involves GTPase Rab5[3] (Rab family is a member of Ras superfamily of G-proteins). Vacuolar acidification is required for release of Wnt protein. Small drug inhibition of V-ATPase a proton pump, prevent WIS from releasing Wnt, so that Wnt-WlS complex accumulates in the cell and plasma membrane.[8]

Wnt proteins do not exit a cell through passive transport or bulk flow but require specific cargo proteins such as the p24 family of highly conserved transmembrane receptors to exit from the ER.[8]

If subunits of retromer components VPS35 and VPS26 are mutant, WIS is targeted for degradation rather than for endosomal recycling to Golgi.[8] Extracellularly, Wnts bind to lipoprotein particles or heparan sulfate proteoglycans or form multimers, which facilitate long range Wnt signaling.[11,12]

WNT INHIBITORS

Wnt function is modulated extracellularly through interaction with a diverse group of secreted Wnt inhibitors that include secreted Frizzled–related protein family (SFRPs), Wnt inhibitor factor (WIF-1), Dkk and Cerberus.

SFRPs, WIF-1 and Cerberus directly bind to Wnt proteins and antagonize Wnt functions by preventing interactions with Frizzled. The secreted Dickkopf proteins inhibit Wnt signaling by direct binding to lipoprotein receptor-related protein 5 and 6 (LRP 5/6). Through this interaction, Dkk crosslinks LRP6 to Kremens, a transmembrane molecule, thus inactivating LRP6.[10] adenomatosis polyposis coli downregulated 1 is a membrane-bound glycoprotein that inhibits Wnt signaling by binding both Wnt and LRP.[9] protein phosphatases (PP2A) also regulate β catenin stability as antagonists of serine kinase. PP2A is required for the elevation of β catenin levels that is dependent on Wnt. Moreover, PP2A can bind to Axin and APC suggesting that it might function to dephosphorylate GSK3 substrates.[10]

WNT SIGNALING PATHWAY

Three different pathways are activated upon Wnt receptor activation:

- Canonical Wnt/β catenin cascade
- Noncanonical planar cell polarity pathway in Drosophila activates small G proteins, including Rac (Ras-related C3 Botulinum toxin substrate – Rhodopsin (Rho) family) and Rho, c-Jun N-terminal kinase and Rho associated kinase. In vertebrates, a counter pathway exists in the convergent extensive movement of tissues during gastrulation and neurulation
- Wnt/Ca^{2+} pathway activates Calcium/Calmodulin–dependent protein kinase II and protein kinase C (Pkc). This pathway has been shown to control the dorsoventral axis patterning in vertebrates.[13]

Although many refer to canonical or noncanonical Wnts this difference is conferred by cellular context as determined by the expression of receptors and signal transducers rather than by an intrinsic property of Wnt proteins. This hypothesis is supported by the fact that noncanonical Wnt5a can act canonically by activating β catenin signaling in certain contexts.[8]

CANONICAL β CATENIN PATHWAY

The canonical Wnt pathway utilizes a very intriguing mechanism when compared to other signaling pathways. Instead of the typical phosphorylation events, cells constantly synthesize and degrade β catenin except in instances when a Wnt signal is present.[14]

Signaling pathway is initiated by Wnt ligand binding to two receptor molecules Frizzled proteins and LRP-5/6.[15]

The Wnt receptor Frizzled signals through the heterotrimeric G proteins. A molecule that directly interacts with F2 is Dsh. LRP contacts the cytoplasmic component and it has been reported that Axin can interact with the cytoplasmic tail of LRP.[16] Wnt signaling leads to the formation of a complex, including Fz, LRP, Axin and Dsh.[16] Dsh transduces the Wnt signal into the cell after direct binding with Fz. Wnt signaling leads to differential phosphorylation of Dsh and this is mediated by a protein kinase protease activated receptor 1. Wnts induce phosphorylation of LRP, thus allowing docking of Axin to LRP cytoplasmic tail. This is followed by a direct

interaction between Axin and Dsh through the Dsh/Axin homologous (DIX) domains.[7]

SIGNALING WITHIN THE CYTOPLASM

The tumor suppressor protein Axin acts as a scaffold of the cytoplasmic destruction complex as it directly interacts with β catenin, tumor suppressor protein APC and the 2 kinases families CK1 and GSK3. When Fz/LRP receptors are not engaged (in the absence of Wnt signal) β catenin binds to Axin. CK1 phosphorylates β catenin at Ser 45. There is subsequent phosphorylation by GSK3 at Ser33, Ser37 and Ser41. This is followed by phosphorylation of APC by CK1 and GSK3. This increases the affinity between APC and β catenin. There is a transfer of β catenin from Axin to APC and Axin binds to the next β catenin molecule. At the final stage, APC exposes N-terminally phosphorylated β catenin to β-TrCP. The ubiquitin ligase is responsible for ubiquitination of β catenin, leading to its degradation in the proteasome.[3]

Activation of Wnt signaling inhibits β phosphorylation and hence its degradation. The elevation of β catenin levels leads to nuclear accumulation and complex formation with Lef/Tcf transcription factors.[16] Two proteins Tcf and Pygopus have been proposed to anchor β catenin in the nucleus, but β catenin can localize in the nucleus in the absence of the two. β catenin can be transported back to the cytoplasm as cargo of Axin or APC. In the absence of Wnt signals, Tcf as a transcriptional repressor by forming complex with Groucho. The interaction of β catenin with N terminus of Tcf converts it into a transcriptional activator for which Groucho is displaced by β catenin from Tcf and histone acetylase CBP/P300 (cyclic AMP response element binding protein/CREB binding protein) is recruited. It has been postulated that CBP binds to β catenin Tcf complex as a coactivator.[10] Brg-1, a component of SNF chromatin remodeling complex, which along with CBP induces chromatin remodeling.[16]

Further interaction between β catenin Tcf complex is mediated by Legless and Pygopus.[16] The overexpression of both these genes has been found to promote Tcf/β catenin activity in mammalian cells. Legless/Bcl9 bridges Pygopus to N-terminus of β catenin. The formation of this trimeric complex has been implicated in retention of β catenin and may also contribute to the ability of β catenin to transactivate transcription.[10]

FRIZZLED

The mammalian genome harbors 10 Fz genes.[11] These receptors, starting from N-terminal have a putative signal sequence followed by a sequence of 120 amino acids containing 10 highly conserved cysteine residues, a highly divergent region of 40–100 amino acids that forms a flexible linker, seven transmembrane segments separated by short extracellular and cytoplasmic loops and a cytoplasmic tail.[17] Two proteins activate Frizzled or LRP receptors-Norrin and R-spondins. Norrin binds to Frizzled-4 and activates canonical signaling pathway in LRP5/6 dependent fashion.[5] It has been shown that R-spondins can physically interact with the extracellular domain of LRP6 and Fz8 and activate Wnt genes.[10]

LIPOPROTEIN RECEPTOR-RELATED PROTEIN 5 AND 6 (LOW DENSITY LIPOPROTEIN LIPOPROTEIN RECEPTOR-RELATED PROTEIN)

In binding Wnt, Frizzled receptors cooperate with a long single pass transmembrane molecule of LRP family known as Arrow in Drosophila and LRP 5 and 6 in vertebrates.[10] The cytoplasmic tail of LRP interacts directly with Axin.[16] LRP 6 plays a dominant role in embryogenesis and LRP 5 in bone homeostasis.[11]

DISHEVELLED

Dsh encodes a ubiquitously expressed cytoplasmic protein containing four domains. At the N terminus, there are 50 amino acids similar to a region in Axin. The other three domains are a short basic domain, centrally located PDZ domain and a more C terminal DEP domain which is also found in several proteins interacting with Pkc.[17] Dsh participates in both β catenin-dependent and independent Wnt signaling.[3]

AXIN

Axin has emerged from studying a classical mouse mutation, fused (now called Axin).[17] Axin serves as a coordinating scaffold for the kinases GSK3 and CK-1 for APC, Dsh and β catenin. There are 2 Axin proteins in humans, Axin 1 and Axin 2. Each of these genes encodes 2 isoforms. Axin 2 expression is upregulated by Wnt/β catenin signaling. Axin has many domains, including a regulators of G protein signaling domain and a DIX domain. The DIX domain forms a heterodimer with Dsh. Axin can be dephosphorylated by serine/threonine phosphatases PP1 and PP2C. Three nuclear localization signals sequences (NLSs) are found in the Axin proteins. Axin lacking NLS fails to regulate cytoplasmic levels of β catenin. It has been suggested that Axin may serve as a shuttle for β catenin between the cytoplasm and the nucleus. It has also been shown that Axin may act as a molecular shuttle to export β catenin from the nucleus.[3]

ADENOMATOUS POLYPOSIS COLI

APC is the largest structural core protein of the destruction complex. APC has been found to act as a nuclear shuttling protein. It has 2 NLSs, which use importin to shuttle APC into the nucleus. APC has many functional domains, including oligomerization domain, 7 armadillo repeats and 3 β catenin binding repeats of 15 amino acids. These repeats have been proposed to bind β catenin and assist in its positioning to the

binding sites of kinases in destruction complex. APC also has seven 20-amino acid repeats involved in the release of β catenin after phosphorylation.[3]

CASEIN KINASE-1

Three casein kinase-1 (CKIs family of kinases) have been implicated in canonical Wnt pathway, CK1α, CK1ε and CK1γ. CK1α binds Axin and phosphorylates β catenin at Ser 45 to prime GSK3 phosphorylation at N terminal residues.[14]

GLYCOGEN SYNTHASE KINASE 3

GSK3 was identified as a serine/threonine protein kinase. Two isoforms are seen in humans GSK3α and GSK3β. GSK3 binds a central region within Axin. A single Axin helix fits into a hydrophobic groove in C terminus of GSK3, leaving GSK3 active site free to phosphorylate β catenin.[14] The release of β catenin from phosphorylation by CK1α and GSK3 may be the only mechanism for Wnt signalosome to regulate β catenin levels. LRP6 can stabilize β catenin indirectly through Axin degradation and GSK3 inhibition. Also induced GSK3 internalization by multivesicular endosomes occurs which reduces β catenin degradation.[3]

β CATENIN AND CADHERINS

Cell adhesion is essential for the regulation of cell behavior. Cadherins are calcium-dependent adhesion molecules. Several forms of cadherins have transmembrane domains while some are attached at the membrane by a phospholipid linkage. They contain several repetitive extracellular domains and function to bring two adjacent cells in close proximity for membrane linked molecular interactions. They have frequently been found to be associated with other co- receptors at the cell surface and influence signaling. β catenin connects cadherins to the actin cytoskeleton during cell-cell adhesion. Cell adhesion mediated by E-cadherin requires, in addition to the availability of calcium ions on the cell surface, the adequate presence of other proteins, such as the so-called α, β and δ catenins present on the cytoplasmic side of the plasma membrane, which anchor E-cadherin to the actin cytoskeleton. α catenin and β catenin bind to cadherins on the intracellular side of the membrane. This helps to enable cadherin based adhesion. Cadherin helps to establish cell polarity and establishes tight junctions. Cadherins promote the formation of cell groups to aid interactions within or between these groups.[18] These associations of classical cadherins with their catenins form structures identified by microscopy as adherent junctions. These molecules play an important role in the maintenance of epithelial tissue architecture, as well as in organogenesis and morphogenesis. E-Cadherin and α catenin, together with β catenin, form a complex that is necessary for cell adhesion.[18]

Catenin has been shown to act both as a regulator of E-cadherin-dependent cell-to-cell adhesion and as an essential mediator in the Wnt-signaling pathway. Experimental data indicate that the presence of β catenin in the cellular junctions is controlled by tyrosine phosphorylation of Tyr- 654, a residue located in the 12th and last armadillo repeat of β catenin, modifies the association of this protein to E-cadherin. The armadillo repeat domain has been shown to be essential for the binding of β catenin to its many binding partners, as E-cadherin and the transcription factor Tcf-4. Piedra *et al.* indicated that phosphorylation of β catenin Tyr-654 increases binding of this protein to TBP both *in vitro* and *in vivo* and this greater association correlates with a higher stimulation of Tcf-4-β catenin transcriptional activity. This higher stimulation of Tcf-4 transcriptional activity observed *in vivo* by beta-catenin Tyr-654 3 Glu mutant is not a consequence of its impaired association to E-cadherin, since it is observed in cells that present very low levels of E-cadherin and their data suggested that phosphorylation of Tyr-654 is relevant not only for disruption of beta-catenin-E-cadherin binding but for stimulation of the interaction of beta-catenin to the basal transcriptional machinery as well.[19]

Bajpai *et al*. demonstrated that β catenin serves as a clutch that facilitates the transition from the low adhesion state of Ecad/Ecad bond to its strong adhesion state. Moreover, although genetic fusion of α catenin to Ecad is enough to strengthen an intracellular Ecad/Ecad bond, the presence of WT β catenin is essential for the cell to respond to altered ECM ligand. Such a stepped control over the strength of Ecad/Ecad bonds allows the cells to modulate their global intercellular adhesion. Such a scenario is ideal for efficient control of global intercellular adhesion both in development as well as in cancer progression.[20]

ODONTOGENESIS AND β CATENIN

Similar to development of many other organs, development of the tooth, from its initiation through morphogenesis to cytodifferentiation, involves a series of sequential and reciprocal signaling interactions between the adjacent epithelium and mesenchyme.

At the beginning of tooth development, the presumptive dental epithelium provides the tooth initiation signals to activate odontogenic potential in the developing tooth mesenchyme. Several signaling molecules, including bone morphogenetic protein (Bmp2), Bmp4, Fgf8, Fgf9, Shh, Wnt4, Wnt6, Wnt10a and Wnt10b, are expressed in the presumptive dental epithelium at the beginning of tooth development. β catenin-mediated canonical Wnt signaling is also required for the activation of odontogenic potential in the developing tooth mesenchyme for tooth development beyond the bud stage. Wnt/β catenin signaling plays a direct and essential role in activation of odontogenic mesenchyme. Several Wnt genes are expressed specifically in the developing dental epithelium during tooth bud formation and exogenous Wnt1, as well as Wnt10b, induce Lef1 mRNA expression in the E11.5 mouse

mandibular mesenchyme suggesting that canonical Wnt signaling may be involved in the epithelial–mesenchymal interactions during tooth initiation.[21]

Chen *et al*. in his study showed that tissue-specific inactivation of β catenin in the developing tooth mesenchyme resulted in cell-autonomous loss of Lef1 mRNA expression, without affecting Bmp4 expression, in the developing tooth mesenchyme in mutant embryos. Thus, in the absence of β catenin mediated canonical Wnt signaling, Bmp4 was insufficient to maintain Lef1 expression in the developing tooth mesenchyme and suggested that Lef1 mRNA expression is directly regulated by Wnt/β catenin signaling in the developing tooth mesenchyme.[21]

A study by Liu *et al*. demonstrated that Wnt/β catenin signaling is active throughout tooth development. A gain of functional mutation in epithelial β catenin results in expanded expression of several key regulatory genes. Conversely, expression of these key dental regulators is disrupted when epithelial, and mesenchymal Wnt/β catenin signaling is inhibited soon after tooth initiation in Dkk1-expressing embryos, resulting in arrested development at the early bud stage. Depletion of epithelial β catenin produces a similar, albeit less severe, phenotype, demonstrating a requirement for epithelial Wnt/β catenin signaling at early stages of tooth development. The stronger phenotypes resulting from ectopic Dkk1 expression compared with β catenin depletion could be due to more efficient and earlier Wnt inhibition in Dkk1-expressing embryos and/or to additional roles for β catenin signaling within mesenchymal cells and they also further demonstrated that inducible Wnt inhibition during molar cusp development results in defective cusp formation and loss of molar tooth polarity.[22]

β catenin signaling is required for embryonic tooth morphogenesis and promotes continuous tooth development when activated in embryos. To determine whether activation of this pathway in the adult oral cavity could promote tooth development, Liu *et al*. induced mutation of epithelial β catenin to a stabilized form in adult mice. This caused increased proliferation of the incisor tooth cervical loop, outpouching of incisor epithelium, the abnormal morphology of the epithelial-mesenchymal junction and enhanced expression of genes associated with embryonic tooth development. Ectopic dental-like structures were formed from the incisor region following implantation into immunodeficient mice. Thus, they concluded that forced activation of β catenin signaling can initiate an embryonic-like program of tooth development in adult rodent incisor teeth.[23]

Dact proteins belong to the Dapper/Frodo protein family and function as cytoplasmic attenuators in Wnt and transforming growth factor (TGFβ) signaling. Dact1 is a potent Wnt signaling inhibitor by promoting degradation of β catenin. Li *et al*. identified a novel role for Dact2 as an inhibitor of the canonical Wnt pathway in embryonic tooth development through its regulation of cell proliferation and differentiation.[24]

The expression of Wnt reporter Axin2-LacZ concentrated around the developing roots, suggesting a high level of Wnt signaling activity in this region. Wnt10a and Wnt10b are highly expressed by differentiating odontoblasts before and during differentiation of root epithelia. A study by Yang *et al*. found that DEpSCs also express Wnt7a and Wnt7b that was upregulated by Alk3 depletion. Hence, multiple Wnt ligands from both dental epithelial and mesenchymal cells might be involved in the differentiation to the root epithelial lineage. The Bmp and Wnt/β catenin pathways interact at multiple levels in various cellular contexts. At early stages of tooth morphogenesis, the Wnt-Bmp feedback circuit accounts for reciprocal epithelial-mesenchymal signaling interactions. Later, during odontoblast differentiation, Smad4-mediated inhibition of Wnt/β catenin signaling controls the fate of cranial neural crest cells. They also indicated that in dental epithelia at the differentiation stage, Bmp signaling inhibits Wnt/β catenin signaling through multiple mechanisms, whereas Wnt/β catenin signaling conversely represses Bmp signaling by induction of secreted inhibitors of Bmp/TGFβ pathways. Bmp signaling has been linked to enamel formation, but its role in root epithelial lineage differentiation is unclear and they also demonstrated that cessation of epithelial Bmp signaling by Bmpr1a depletion during the differentiation stage switched differentiation of crown epithelia into the root lineage and led to formation of ectopic cementum-like structures. This phenotype is related to the upregulation of Wnt/β catenin signaling and epithelial-mesenchymal transition (EMT). Although epithelial β catenin depletion during the differentiation stage also led to variable enamel defect and precocious/ectopic formation of fragmented root epithelia in some teeth, it did not cause ectopic cementogenesis and inhibited EMT in cultured dental epithelia. Concomitant epithelial β catenin depletion rescued EMT and ectopic cementogenesis caused by Bmpr1a depletion and suggested that Bmp and Wnt/β catenin pathways interact antagonistically in dental epithelia to regulate the root lineage differentiation and EMT. These findings will aid in the design of new strategies to promote functional differentiation in the regeneration and tissue engineering of teeth and will provide new insights into the dynamic interactions between the Bmp and Wnt/β catenin pathways during cell fate decisions.[25]

Kim *et al*. suggested the role of miR-663 in promoting the differentiation of muscle-derived progenitor cell-23 cells to odontoblasts by targeting APC-mediated activation of Wnt/β catenin signaling and miR-663 can be considered a critical regulator of odontoblast differentiation and can be utilized for developing miRNA-based therapeutic agents.[26]

ENAMEL AND β CATENIN

During tooth development, high levels of nuclear β catenin are observed at the early cap stage, and β catenin mRNA expression is up-regulated in IEE cells and enamel knots.[27]

Obara *et al*. investigated the intracellular distribution of β catenin and E-cadherin in tooth germs in the cap stage and showed variations in β catenin staining among different regions of the enamel organ, with strong staining being detected in the cytoplasm and nucleus of cells located in the enamel knot and the inner epithelium of the enamel organ. Those authors suggested that nuclear staining for β catenin was due to specific activation of the Wnt signaling pathway during this stage of development.[28]

DENTINE AND PULP AND β CATENIN

Dental pulp is a specialized connective tissue that maintains dentin homeostasis, sensation, nutrition and defense. In clinically relevant insults due to trauma or infection, pulp has some ability to form reparative dentin. The differentiation of dental pulp cells probably plays a key role in homeostasis and pulp/dentin regeneration. Dental pulp cells can differentiate into odontoblast-like cells, which are thought to be responsible for secreting the dentin matrix and forming reparative dentin, making it possible to retain the vitality of dental pulp by clinical therapies. A variety of factors participate in the regulation of dental pulp cell differentiation, including specific proteins and cytokines such as BMP, dentin sialophosphoprotein and dentin matrix protein-1. Yang *et al*. demonstrated that BMP2 promotes the differentiation of human dental pulp cells by activating WNT/β catenin signaling, which is further mediated by p38 mitogen-activated protein kinase (MAPK) *in vitro* and their findings indicated a potential mechanism by which BMP2 regulates WNT/β catenin, thereby mediating cell differentiation and dentin formation in the pulp repair process.[29]

Kim *et al*. demonstrated in their study that persistent stabilization of beta-catenin in the dental mesenchyme leads to premature differentiation of odontoblasts and differentiation of cememtoblasts and induces excessive dentine and cementum formation *in vivo* and suggested that temporospatial regulation of Wnt/β catenin signaling plays critical roles in odontoblast and cementoblast differentiation and that inhibition of Wnt/β catenin signaling is important for the formation of dentin and cementum during tooth development.[30]

Zhang *et al*. in their experimental study found that ablation of β catenin in the odontoblasts and cementoblasts severely disrupted the morphogenesis of the molar roots and the root analogue of incisor and their study provided a strong evidence that the integrity of Wnt/β catenin signaling pathway within odontoblasts is of great importance to cell differentiation and proliferation during tooth root development. As for a tooth root, the most important construction is the fine-deposited dentin produced by root odontoblasts. In OC-Cre;Ctnnb1fl/fl mice, β catenin was ablated from preodontoblasts, resulting in the blockade of odontoblastic differentiation and the reduction of cellular proliferation, thus arrested root dentin synthesis, and ultimately leading to the absence of tooth root. However, in crown odontoblasts, which have finished their differentiation in embryo, following knockout of β catenin has no visible effect on their further maturation or maintenance. Wnt/β catenin signaling has a positive stimulating effect on dental papilla stem cells on aspects of proliferation and differentiation, further confirming that Wnt/β catenin signaling dominates odontogenesis via regulating their odontoblastic differentiation. Constitutive stabilization of β catenin in developing odontoblasts leads to abnormal teeth as characterized by shortened roots with odontoblasts acceleratedly differentiated and the resulting excessively deposited dentin and they indicated that Wnt/β catenin signaling is of significant importance in tooth root development by promoting the differentiation from mesenchyme to odontoblasts.[31]

Wang *et al*. showed that Dkk1 negatively regulated canonical Wnt signaling and blocked odonto/osteogenic differentiation, which completely prevented Wnt1-mediated odontogenic events from occurring and suggested a positive role for Wnt/β catenin in differentiation and mineralization of stem cells from the apical papilla (SCAP) and their study supported the idea that Wnt/β catenin significantly promotes SCAP proliferation and odonto/osteogenic differentiation.[32]

A study by Scheller *et al*., indicated that canonical Wnt/β catenin signaling was sufficient to suppress differentiation and mineralization of dental pulp stem cells (DPSCs), maintaining DPSCs in an undifferentiated state. Cheng *et al*., in their study concluded that β catenin and Rho make a major contribution to dental pulp repair. Shao *et al*. speculated that TGF-β1 is released from pulp cells and the ECM activates Rho GTPases to facilitate cell motility during early injury to dental pulp. Simultaneously, Rho cross-talk with Wnt/β catenin restrains cell differentiation. When cells migrate to the site of injury, TGF-β1 interplays with other signaling pathways (e.g. MAPK) via Smads to promote cell differentiation and proliferation. So far, only a few reports give emphasis on the area for pathways serving functions in pulp repair.[33]

Wnt/β catenin signaling stimulates bone formation by enhancing osteoblast differentiation, suggest that activation of the Wnt signaling pathway may associate with the regeneration of dental hard tissues, dentin and cementum, as well as a bone. For example, stabilization of β catenin in the dental epithelium is associated with mesenchymal expression of signaling molecules such as Bmp4, Bmp2, Bmp7, fibroblast growth factor-3 (Fgf3), activin and follistatin. Further, Wnt10a has been implicated as a key molecule for dentinogenesis, acting as a regulator of cell-matrix interactions during odontoblast differentiation. Inhibition of canonical Wnt signaling, either by deleting Lef1 function or over-expressing the Wnt inhibitor Dkk1, arrests tooth morphogenesis at an early stage of tooth development and inactivating familial mutations in Axin2, a negative regulator of β catenin, causes decreased tooth number because of the lack of tooth renewal. Conversely, activation

of the Wnt/β catenin pathway by exogenous expression of active Ctnnb1, promotes the ectopic formation of the tooth, following transplantation to a kidney capsule.[34]

DENTAL FOLLICLE CELLS AND β CATENIN

Periodontal ligament cells (PDLCs) have the capacity *in vitro* to differentiate into osteoblasts and cementoblasts due to the multilineage differentiation potential. The cementogenic differentiation of PDLCs is spatially and temporally regulated by the cell signaling proteins and genes. The study by Han *et al*. showed that 5% lithium (Li) ions incorporated into mesoporous bioactive glass scaffolds enhanced the proliferation and cementogenic differentiation of hPDLCs on scaffolds, most likely via activation of Wnt/β catenin signaling pathway and their study demonstrated that Li ions by themselves significantly enhanced the proliferation, differentiation and cementogenic gene expression of PDLCs and their results indicated that incorporation of Li ions into bioactive scaffolds is a viable means of enhancing the Wnt canonical signaling pathway to stimulate cementogenic differentiation of PDLCs.[35]

Dental follicle cells (DFCs) are reported to contain stem cells. The canonical Wnt signaling pathway plays an important role in stem cell self-renewal and tooth development through β catenin expression. Wnt/β catenin signaling regulates the proliferation or differentiation of cementoblast/osteoblast from the cultured DFCs. Du *et al*. suggested that Wnt/β catenin signaling pathway positively regulates the cementoblast/osteoblast differentiation of the DFCs.[36]

Cyclin D1, a target gene of the β catenin/Lef1 pathway is a major regulator of the progression of cells into the proliferative stage of the cell cycle. Nemoto *et al*. demonstrated that Wnt3a induced the expression of cyclin D1 by cementoblasts accompanied by an increased proliferative response. Cyclin D1-cell division kinase 4 induces Runx2 phosphorylation, ubiquitination and proteasome degradation and thereby inhibits differentiation while stimulating proliferation. It is possible that inhibition of cementoblast differentiation mediated by Wnt3a may be a result of Wnt3a's ability to directly reduce Runx2 expression as well as its ability to induce cyclin D1 expression resulting in a further decrease in Runx2 expression. In conclusion, these observations suggest that Wnt signaling inhibits cementoblast differentiation and promotes cell proliferation. Elucidating the role of Wnt in controlling cementoblast function will provide new tools needed to improve on existing periodontal regeneration therapies.[37]

Wnt/β catenin signaling is known to be crucial for tooth development and bone formation. For instance, depletion of β catenin in the oral mesenchyme causes the arrest of tooth development at the bud stage. Mesenchymal β catenin was also required for the expression of LEF1 and Fgf3 in the dental mesenchyme and the induction of the primary enamel knot in the developing epithelium. Furthermore, several members of the Wnt family were observed in the presumptive dental epithelium or mesenchyme. Canonical Wnt/β catenin signaling pathway increases bone mass through a number of mechanisms including renewal of stem cells, stimulation of preosteoblast replication, induction of osteogenesis and inhibition of osteoblast and osteocyte apoptosis, suggesting that activation of the Wnt signaling pathway may assist in regeneration of bone and associated periodontal tissues.[38]

REFERENCES

1. Valenta T, Hausmann G, Basler K. The many faces and functions of ß-catenin. EMBO J 2012;31:2714-36.
2. Daugherty RL, Gottardi CJ. Phospho-regulation of Beta-catenin adhesion and signaling functions. Physiology (Bethesda) 2007;22:303-9.
3. Voronkov A, Krauss S. Wnt/beta-catenin signaling and small molecule inhibitors. Curr Pharm Des 2013;19:634-64.
4. Zhao ZM, Reynolds AB, Gaucher EA. The evolutionary history of the catenin gene family during metazoan evolution. BMC Evol Biol 2011;11:198.
5. Xu W, Kimelman D. Mechanistic insights from structural studies of beta-catenin and its binding partners. J Cell Sci 2007;120 (Pt 19):3337-44.
6. Xing Y, Takemaru K, Liu J, Berndt JD, Zheng JJ, Moon RT, *et al.* Crystal structure of a full-length beta-catenin. Structure 2008;16:478-87.
7. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781-810.
8. Willert K, Nusse R. Wnt proteins. Cold Spring Harb Perspect Biol 2012;4:a007864.
9. Clevers H, Nusse R. Wnt/ß-catenin signaling and disease. Cell 2012;149:1192-205.
10. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127:469-80.
11. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: Components, mechanisms and diseases. Dev Cell 2009;17:9-26.
12. Miller JR, Hocking AM, Brown JD, Moon RT. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca^{2+} pathways. Oncogene 1999;18:7860-72.
13. Kurayoshi M, Yamamoto H, Izumi S, Kikuchi A. Post-translational palmitoylation and glycosylation of Wnt-5a are necessary for its signalling. Biochem J 2007;402:515-23.
14. Kimelman D, Xu W. Beta-catenin destruction complex: Insights and questions from a structural perspective. Oncogene 2006;25:7482-91.
15. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin and cadherin pathways. Science 2004;303:1483-7.
16. Nusse R. Wnt signaling in disease and in development. Cell Res 2005;15:28-32.
17. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol 1998;14:59-88.
18. Alves Pereira KM, do Amaral BA, dos Santos BR, Galvão HC, Freitas Rde A, de Souza LB. Immunohistochemical expression of E-cadherin and beta-catenin in ameloblastomas and tooth germs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:425-31.
19. Piedra J, Martinez D, Castano J, Miravet S, Dunach M, de Herreros AG. Regulation of beta-catenin structure and activity

by tyrosine phosphorylation. J Biol Chem 2001;276:20436-43.
20. Bajpai S, Feng Y, Wirtz D, Longmore GD. ß-Catenin serves as a clutch between low and high intercellular E-cadherin bond strengths. Biophys J 2013;105:2289-300.
21. Chen J, Lan Y, Baek JA, Gao Y, Jiang R. Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. Dev Biol 2009;334:174-85.
22. Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, *et al.* Wnt/beta-catenin signaling directs multiple stages of tooth morphogenesis. Dev Biol 2008;313:210-24.
23. Liu F, Dangaria S, Andl T, Zhang Y, Wright AC, Damek-Poprawa M, *et al.* Beta-Catenin initiates tooth neogenesis in adult rodent incisors. J Dent Res 2010;89:909-14.
24. Li X, Florez S, Wang J, Cao H, Amendt BA. Dact2 represses PITX2 transcriptional activation and cell proliferation through Wnt/beta-catenin signaling during odontogenesis. PLoS One 2013;8:e54868.
25. Yang Z, Hai B, Qin L, Ti X, Shangguan L, Zhao Y, *et al.* Cessation of epithelial Bmp signaling switches the differentiation of crown epithelia to the root lineage in a ß-catenin-dependent manner. Mol Cell Biol 2013;33:4732-44.
26. Kim JS, Park MG, Lee SA, Park SY, Kim HJ, Yu SK, *et al.* Downregulation of adenomatous polyposis coli by microRNA-663 promotes odontogenic differentiation through activation of Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2014;446:894-900.
27. Otsu K, Kishigami R, Fujiwara N, Ishizeki K, Harada H. Functional role of Rho-kinase in ameloblast differentiation. J Cell Physiol 2011;226:2527-34.
28. Obara N, Lesot H. Subcellular localization of beta-catenin and cadherin expression in the cap-stage enamel organ of the mouse molar. Histochem Cell Biol 2004;121:351-8.
29. Yang J, Ye L, Hui TQ, Yang DM, Huang DM, Zhou XD, *et al.* Bone morphogenetic protein 2-induced human dental pulp cell differentiation involves p38 mitogen-activated protein kinase-activated canonical WNT pathway. Int J Oral Sci 2015;7:95-102.
30. Kim TH, Lee JY, Baek JA, Lee JC, Yang X, Taketo MM, *et al.* Constitutive stabilization of ß-catenin in the dental mesenchyme leads to excessive dentin and cementum formation. Biochem Biophys Res Commun 2011;412:549-55.
31. Zhang R, Yang G, Wu X, Xie J, Yang X, Li T. Disruption of Wnt/ß-catenin signaling in odontoblasts and cementoblasts arrests tooth root development in postnatal mouse teeth. Int J Biol Sci 2013;9:228-36.
32. Wang J, Liu B, Gu S, Liang J. Effects of Wnt/ß-catenin signalling on proliferation and differentiation of apical papilla stem cells. Cell Prolif 2012;45:121-31.
33. Shao MY, Cheng R, Wang FM, Yang H, Cheng L, Hu T. ß-Catenin and Rho GTPases as downstream targets of TGF-ß1 during pulp repair. Cell Biol Int 2011;35:105-9.
34. Silvério KG, Davidson KC, James RG, Adams AM, Foster BL, Nociti FH Jr, *et al.* Wnt/ß-catenin pathway regulates bone morphogenetic protein (BMP2)-mediated differentiation of dental follicle cells. J Periodontal Res 2012;47:309-19.
35. Han P, Wu C, Chang J, Xiao Y. The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/ß-catenin signalling pathway by Li+ions released from bioactive scaffolds. Biomaterials 2012;33:6370-9.
36. Du Y, Ling J, Wei X, Ning Y, Xie N, Gu H, *et al.* Wnt/ß-catenin signaling participates in cementoblast/osteoblast differentiation of dental follicle cells. Connect Tissue Res 2012;53:390-7.
37. Nemoto E, Koshikawa Y, Kanaya S, Tsuchiya M, Tamura M, Somerman MJ, *et al.* Wnt signaling inhibits cementoblast differentiation and promotes proliferation. Bone 2009;44:805-12.
38. Cao Z, Liu R, Zhang H, Liao H, Zhang Y, Hinton RJ, *et al.* Osterix controls cementoblast differentiation through downregulation of Wnt-signaling via enhancing DKK1 expression. Int J Biol Sci 2015;11:335-44.