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ABSTRACT

The Wnt/ β -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/ β -catenin signaling promotes the formation of ectopic teeth and taste papillae. In this review, we discuss our current understanding of the roles of Wnt/ β -catenin signaling in oral tissue development and in related human diseases, and the potential of manipulating this pathway for therapeutic purposes.

KEY WORDS: Wnt, β -catenin, development, tooth, taste, lip, oral tissue, cancer.

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Wnt/ β -catenin Signaling in Oral Tissue Development and Disease

INTRODUCTION

Oral diseases present major and prevalent public health problems, and oral cancers can be life-threatening. Elucidation of the molecular mechanisms regulating the development and regeneration of oral tissues will provide insight into the etiology underlying these disorders, and has the potential to identify novel therapeutic targets, as well as to contribute to regenerative medicine. Recent advances in oral tissue research have revealed essential roles for the Wnt/ β -catenin signaling pathway in the development of many oral tissues.

THE WNT/ β -CATENIN PATHWAY

WNT paracrine signaling molecules form a family of evolutionarily conserved secreted glycoproteins with at least 19 members in humans and mice (Mikels and Nusse, 2006). WNT proteins signal across cell membranes by interacting with serpentine receptors of the Frizzled (Fzd) family. WNT ligands activate several known pathways: the Wnt/ β -catenin or canonical pathway; the Wnt/ Ca^{+2} pathway involving Protein Kinase A; the planar cell polarity pathway; and a pathway involving Protein Kinase C that functions in muscle myogenesis (Gordon and Nusse, 2006). The Wnt/ β -catenin pathway is the best-studied of these. Membrane β -catenin is a component of intercellular adhesion junctions, where it connects cadherins to α -catenin and forms a dynamic link to the cytoskeleton (Drees *et al.*, 2005; Yamada *et al.*, 2005). In the absence of canonical Wnt signaling, cytoplasmic β -catenin is associated with adenomatous polyposis coli (APC) and Axin proteins, and is phosphorylated by glycogen synthase kinase 3 β (GSK3 β) and casein kinase I (CKI) in its N-terminal degradation box, resulting in polyubiquitination by β TRCP1 or β TRCP2 complexes and targeting for protease-mediated degradation (Liu *et al.*, 1999). Binding of WNT ligand to a Frizzled (FZD) receptor and a low-density lipoprotein-related-receptor protein (LRP) 5 or 6 co-receptor, and interaction of FZD with the cytoplasmic protein Disheveled (DSH), result in phosphorylation of cytoplasmic DSH by CKI and its binding to GSK3 β with the help of Frequently Rearranged in Advanced T-cell lymphomas (FRAT) protein. These interactions cause inactivation of the Axin/APC/GSK3 β /CKI complex and stabilization of cytoplasmic β -catenin. A novel member of the FRL1/Crypto class of receptors can also mediate Wnt/ β -catenin signaling triggered by Wnt11, together with Frizzled in *Xenopus* embryos (Tao *et al.*, 2005).

Nuclear β -catenin interacts with members of the Lymphoid Enhancer Factor/T-cell factor (LEF/TCF) family of transcription factors and also with Legless family docking proteins BCL9/BCL9L, which associate with the transcription co-activator pygopus (PYGO) 1/2 (Kramps *et al.*, 2002). The

LEF/TCF/ β -catenin complex recruits transcription co-activator CREB binding protein (CBP) and the closely related protein p300. This complex activates the transcription of target genes. β -catenin also binds to the TATA box-binding protein (TBP), either directly or through another factor, TIP49 (also known as Pontin52) (Bauer *et al.*, 2000), thus connecting to the basal transcription machinery.

Traditionally, WNT proteins have been divided into two groups based on their ability to activate Wnt/ β -catenin signaling: Members of the Wnt1 class (WNT1, WNT3, WNT3A, WNT7A, WNT7B, WNT8A) have been considered effective activators of the canonical pathway, whereas members of the Wnt5a class (WNT4, WNT5A, and WNT11) have been considered poor activators or even inhibitors of the canonical pathway (Maye *et al.*, 2004). However, analysis of recent data indicates that the outcome of

signaling is regulated by specific Wnt-receptor pairings rather than being intrinsic to a particular Wnt. For instance, WNT11 and WNT5A are able to signal *via* β -catenin in a Frizzled-FRL- or Frizzled4- and LRP5-dependent manner, respectively (Tao *et al.*, 2005; Mikels and Nusse, 2006).

Multiple secreted and intracellular inhibitors control activity of the Wnt/ β -catenin signaling pathway. Among the secreted Wnt inhibitors, Dickkopf (Dkk) family members interact with LRP5/LRP6 co-receptors and cause endocytosis of the WNT/LRP5/6 complex, while SFRP (secreted Frizzled related protein) family members and WIF1 (Wnt inhibitory factor 1) inhibit binding of WNT ligands to FZD family receptors (Kawano and Kypta, 2003). In addition to components of the β -catenin destruction complex, intracellular inhibitors include the Dishevelled-binding protein NKD (naked cuticle) (Wharton *et al.*, 2001), and nemo-like kinase (NLK), which phosphorylates LEF/TCF proteins and inhibits the DNA-binding ability of β -catenin/TCF complexes (Ishitani *et al.*, 1999). A simplified version of the canonical Wnt signaling pathway is shown in Fig. 1; for a more comprehensive survey of canonical Wnt signaling components, see www.stanford.edu/~rnusse/pathways/cell2.html.

WNT/ β -CATENIN SIGNALING IN THE DEVELOPMENT OF THE CRANIOFACIAL SKELETON AND MUSCLES

Wnt/ β -catenin signaling is activated in a dynamic fashion in cranial neural crest cells (NCCs) that contribute extensively to forming craniofacial structures (Mani *et al.*, 2010). NCCs

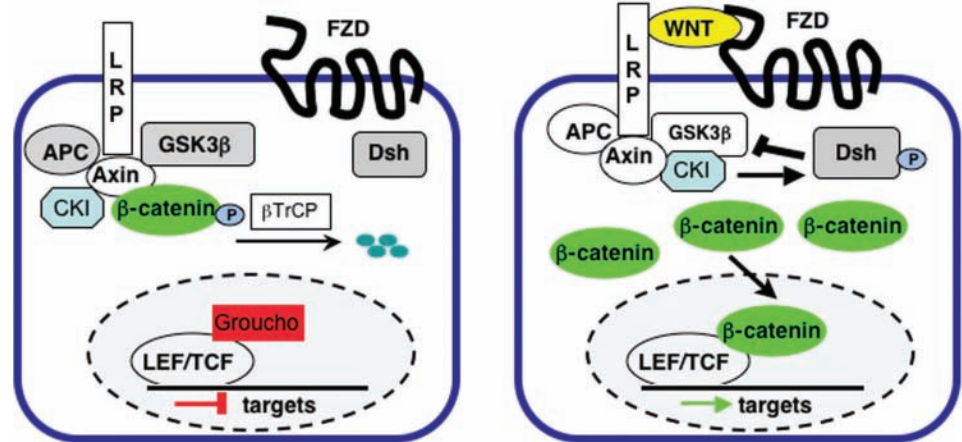


Figure 1. Simplified schematic of the Wnt/ β -catenin signaling pathway. **(Left)** In the absence of a WNT ligand, cytoplasmic β -catenin is associated with APC and Axin, phosphorylated by GSK3 β and CKI, and polyubiquitinated by the β TRCP complex, targeting it for proteosomal degradation; under these conditions, LEF/TCF transcription factors in the nucleus associate with transcriptional co-repressors, such as Groucho, and the transcription of Wnt target genes is repressed. **(Right)** In the presence of a WNT ligand, phosphorylation and degradation of β -catenin are inhibited, allowing it to accumulate in the cytoplasm and translocate into the nucleus. Nuclear β -catenin interacts with LEF/TCF family transcription factors and several other transcriptional co-activators to initiate transcription of target genes. Abbreviations: FZD, Frizzled; LRP, low-density lipoprotein-receptor-related protein; APC, adenomatous polyposis coli; GSK, glycogen synthase kinase; CKI, casein kinase I; DSH, Dishevelled; LEF/TCF, Lymphoid enhancer factor/T-cell factor.

migrate into the first branchial arch from the midbrain and anterior hindbrain at around the four-somite stage (Serbedzija *et al.*, 1992). These cells will form the skeleton of the upper and lower jaws, and contribute to the trigeminal ganglion (Tan and Morriss-Kay, 1985). In neural-crest-specific β -catenin knockout mice, these cranial NCC-derived structures are absent, and apoptosis is increased in cells migrating to the cranial ganglia and in areas of prechondrogenic condensations, indicating the pivotal role of Wnt/ β -catenin signaling in neural crest cell survival and/or differentiation (Brault *et al.*, 2001).

Wnt/ β -catenin signaling also plays essential roles in general skeletal development. Skeletons develop by two processes: intramembranous and endochondral ossification. The majority of skeletons are formed by endochondral ossification, whereby mesenchymal cells condense and cells within the condensations differentiate into chondrocytes, forming a cartilaginous template that prefigures the future skeletal element, while the surrounding cells form the perichondrium. In micromass cultures in the mouse and chicken, Wnt/ β -catenin signaling represses chondrogenesis in limb buds and in the craniofacial region by repressing *Sox9* transcription (Cheung *et al.*, 2005; Hill *et al.*, 2005; Später *et al.*, 2006). Wnt signaling also controls chondrocyte maturation. Overexpression of *Wnt4*, *Wnt8*, or a stabilized form of β -catenin in chicken embryos results in acceleration of chondrocyte maturation (Hartmann and Tabin, 2000; Enomoto-Iwamoto *et al.*, 2002). Conversely, overexpression of a Wnt-antagonist, SFRP3 (FRZB1), results in delayed chondrocyte differentiation, by antagonizing β -catenin-mediated maturation of hypertrophic chondrocytes (Enomoto-Iwamoto *et al.*, 2002), while loss of

SFRP1 results in acceleration of chondrocyte hypertrophy and mineralization (Gaur *et al.*, 2006).

About 40 skeletal muscles are present in the vertebrate head, and most of them (the “genuine” head muscles) develop from the paraxial and prechordal mesoderm located in the preotic levels of the head. Although the genuine head muscles eventually exhibit the same tissue architecture as muscles in the trunk, their development is remarkably distinct and is regulated by different molecular mechanisms (Carvajal *et al.*, 2001). The cranial muscle anlagen in the chick are surrounded by NCCs and other tissues secreting the Wnt ligands WNT1, WNT3A, and WNT13, and the Wnt inhibitor FRZB. Interestingly, although myogenesis in trunk paraxial mesoderm is induced by Wnt/ β -catenin signaling from the dorsal neural tube, myogenesis in cranial paraxial mesoderm is blocked by the same signal; conversely, skeletal myogenesis in the head is induced by the Wnt inhibitor FRZB in combination with the Bone Morphogenetic Protein (BMP) inhibitors Noggin and Gremlin (Tzahor *et al.*, 2003).

THE WNT/ β -CATENIN PATHWAY IN TOOTH DEVELOPMENT

Tooth development is regulated by reciprocal interactions between the dental epithelium and mesenchyme through a complex signaling network (Thesleff, 2006). Tooth development is initiated with thickening of the ectodermally derived oral epithelium. The epithelium invaginates into mesenchyme that originates from the neural crest of the first branchial arch, and forms a tooth bud. Morphogenesis from the bud to the cap stage involves the formation of a transient signaling center at the base of the epithelial bud, termed an “enamel knot”, which expresses multiple signaling molecules, and the development of a mesenchymal papilla. The single primary enamel knot disappears at the bell stage. At this stage in developing molar teeth, several secondary enamel knots are formed, which determine the locations and shapes of the molar cusps. Subsequent differentiation gives rise to dentin-secreting odontoblasts of mesenchymal origin, and enamel-secreting ameloblasts derived from the inner dental epithelium (Jernvall and Thesleff, 2000). Wnt/ β -catenin signaling is dynamically active in tooth-forming regions at all of these stages of tooth development, and plays multiple roles in these events (Jarvinen *et al.*, 2006; Liu *et al.*, 2008).

(1) Initiation Stage

Multiple Wnt genes are expressed at the initiation of mouse tooth development. These include *Wnt10b*, which is expressed specifically in the molar and incisor dental epithelial thickenings, and *Wnt5a* and the Wnt agonist/antagonists *MFrzb1* and *Mfrp2* that are expressed in a graded proximo-distal (P-D) manner in mesenchymal cells, with no overlying expression in the oral or dental epithelium (Sarkar and Sharpe, 1999). At this same stage, expression of *Wnt4*, *Wnt6*, and the Wnt receptor gene *Fz6* is observed in facial, oral, and dental epithelium. Tissue recombination experiments revealed that epithelial *Wnt4* induces expression of *Sema3a*, a secreted repulsive axon guidance molecule, in the presumptive dental mesenchyme, which in turn guides the arrival

of the first dental nerve fibers (Kettunen *et al.*, 2005). *Wnt3* and *Wnt7b* are expressed in the oral epithelium, but are absent from the presumptive dental epithelium. Ectopic expression of *Wnt7b* in presumptive dental ectoderm in mandibular arch explants down-regulates *Sonic Hedgehog* (*Shh*) expression in the presumptive dental ectoderm and the *Shh* effector and target gene *Patched* in the underlying ectomesenchyme, and tooth development is subsequently arrested, suggesting that the expression of *Wnt7b* in presumptive oral ectoderm and *Shh* in presumptive dental ectoderm positions the sites of tooth formation (Sarkar *et al.*, 2000). However, the signaling pathway activated by ectopic *Wnt7b* in these experiments was not identified. Activity of the canonical Wnt/ β -catenin pathway in developing molar teeth *in vivo* has been monitored by immunostaining for nuclear β -catenin, by the use of Wnt reporter transgenes that contain multimerized LEF/TCF binding sites upstream of a minimal promoter and a *lacZ* reporter (*TOPGAL*, *BAT-gal*, and *Lef/Tcf-lacZ*), and by means of a knockin of *lacZ* into the endogenous *Axin2* locus, a direct Wnt target gene (Liu *et al.*, 2008; Lohi *et al.*, 2010). While expression patterns vary slightly for different reporters, these studies, taken together, indicate that Wnt/ β -catenin signaling localizes specifically to the epithelium of the dental lamina and forming dental placodes at the initiation stage of tooth development (Fig. 2), suggesting that it plays a positive, rather than inhibitory, role in dental initiation. This hypothesis is supported by observations of continuous ectopic dental development in embryos bearing an activating, stabilizing mutation in epithelial β -catenin, or homozygous for a null mutation in the intracellular Wnt antagonist APC (Jarvinen *et al.*, 2006; Kuraguchi *et al.*, 2006; Liu *et al.*, 2008).

(2) Bud Stage

At the bud stage, Wnt/ β -catenin reporter gene expression reveals that Wnt/ β -catenin signaling is active in dental epithelial cells and immediately underlying mesenchymal cells (Liu *et al.*, 2008; Lohi *et al.*, 2010) (Fig. 2). When dental epithelial and mesenchymal Wnt/ β -catenin signaling is inhibited soon after tooth initiation by forced epithelial expression of the secreted Wnt inhibitor DKK1, tooth development is arrested at the early bud stage (Liu *et al.*, 2008). 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. Arrested dental development in *Dkk1*-expressing embryos appears to result from loss of expression of *Bmp4* and its target genes *Msx1* and *Msx2*, consistent with the similar tooth phenotypes observed in *Dkk1*-expressing and *Msx1*^{-/-}; *Msx2*^{-/-} embryos (Bei and Maas, 1998; Liu *et al.*, 2008). Deletion of β -catenin in oral mesenchyme results in failure of progression from the bud to the cap stage of tooth development. Mesenchymal β -catenin is required for expression of *Lef1* and *Fgf3* in the dental mesenchyme and for induction of the primary enamel knot in the epithelium (J Chen *et al.*, 2009). These data indicate essential roles for β -catenin signaling within both epithelial and mesenchymal compartments of the developing tooth. Interestingly, the Wnt-activated populations in developing dental epithelium and mesenchyme directly abut each

other, suggesting intimate crosstalk between these compartments that likely involves *Wnt10a*, *Wnt10b*, *Wnt6*, and *Wnt3*, expressed in the primary enamel knot, and *Wnt5a*, expressed in adjacent mesenchyme.

β -catenin and *Lef1* expression in the dental epithelium of developing tooth buds overlaps with expression of PITX2, an essential transcription factor for tooth development (Hjalt *et al.*, 2000). Expression of *Lef1* is regulated through direct physical interactions of PITX2, LEF1, and β -catenin (Vadlamudi *et al.*, 2005), and PITX2 enhances endogenous expression of the full-length β -catenin-dependent *Lef1* isoform while decreasing expression of the N-terminally truncated β -catenin-independent isoform (Amen *et al.*, 2007). Targeted inactivation of the *Lef1* gene in mice results in arrested tooth development at the bud stage (van Genderen *et al.*, 1994), slightly later than the block to tooth development seen in *Dkk1*-expressing embryos. The difference in the stage of arrest is likely due to partially redundant functions of LEF1 with other LEF/TCF family members in tooth development. Recombinations of epithelial and mesenchymal tissues from developing teeth of wild-type and *Lef1*^{-/-} embryos showed that LEF1 is required only transiently in the epithelium in a tissue-non-autonomous manner (Kratochwil *et al.*, 1996). The *Fgf4* gene is a direct transcriptional target of LEF1, and in turn induces expression of *Fgf3* in dental mesenchyme followed by *Shh* in the epithelium, indicating sequential and reciprocal communication between the epithelium and mesenchyme (Kratochwil *et al.*, 2002). Loss of *Lef1* also causes failure of survival of dental epithelial cells (Sasaki *et al.*, 2005). Conversely, overexpression of *Lef1* in the epithelium can trigger the epithelial cells in the lip furrow of developing transgenic animals to invaginate, sometimes leading to inappropriate adoption of hair follicle and tooth cell fates (Zhou *et al.*, 1995).

(3) Cap Stage

At the early cap stage, *Lef1*, *Wnt3*, *Wnt6*, *Wnt10b*, and *MFz6* are detected in the primary enamel knot, a transient signaling center in the developing tooth, while *Wnt5a* and *MFrzb1* show strong expression in the dental papilla mesenchyme (Sarkar and Sharpe, 1999). Conversely, expression of Wnt inhibitors of the *Dkk* family is up-regulated in developing teeth and surrounding oral mesenchyme, but is excluded from enamel knots (Fjeld *et al.*, 2005). Consistent with these data, expression of Wnt/ β -catenin reporter genes and immunostaining for nuclear β -catenin indicate that Wnt/ β -catenin signaling is active in the enamel knot (Liu *et al.*, 2008; Lohi *et al.*, 2010) (Fig. 2), and that β -catenin mRNA is also prominently up-regulated in this structure (Obara *et al.*, 2006; Liu *et al.*, 2008). In mutant embryos carrying an activating, stabilizing mutation in epithelial β -catenin, supernumerary teeth develop by a process in which new enamel knots bud off from the existing dental epithelium, suggesting β -catenin

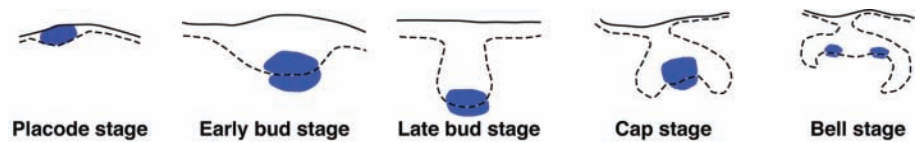


Figure 2. The Wnt/ β -catenin signaling pathway is activated in a dynamic fashion in molar tooth development. Summary of data from analyses of Wnt/ β -catenin pathway activity in developing molar teeth *in vivo* with immunostaining for nuclear β -catenin; X-gal staining of embryos carrying Wnt reporter transgenes that contain multimerized LEF/TCF binding sites upstream of a minimal promoter and a *lacZ* reporter (*TOPGAL*, *BAT-gal*, and *Lef/Tcf-lacZ*); and X-gal staining of embryos in which *lacZ* has been inserted into the endogenous *Axin2* locus, a direct Wnt target gene (Liu *et al.*, 2008; Lohi *et al.*, 2010). While expression patterns vary slightly for different reporters, these studies, taken together, indicate that Wnt/ β -catenin signaling localizes specifically to the epithelium of the dental lamina at the placode stage; to the primary epithelial enamel knot and underlying mesenchymal cells at the bud and cap stages; and to the secondary enamel knots and underlying mesenchymal cells at the bell stage. Wnt/ β -catenin pathway activity is indicated by grey or blue shading in the print and online versions, respectively. Dashed black lines indicate epithelial-mesenchymal borders. Diagrams are not to scale.

signaling as an upstream activator of enamel knot formation (Jarvinen *et al.*, 2006).

(4) Bell Stage

The secondary enamel knots provide the earliest morphologic indication of species-specific cusp patterns. Cusp patterning is thought to begin in molar teeth of mice and voles at the late cap stage, prior to morphological evidence of cusp development (Keranen *et al.*, 1998). Thus, the molecular signals regulating the relative positions of cusps are predicted to be active immediately after apoptosis of the primary enamel knot. In line with this expected pattern, Wnt/ β -catenin signaling is active in developing secondary enamel knots (Liu *et al.*, 2008; Lohi *et al.*, 2010) (Fig. 2). Treatment of molar tooth germ explants with the Wnt inhibitor MFRZB1, followed by transplantation to renal capsules for further development, causes teeth to be reduced in size and with blunter cusps than controls, suggesting that Wnt signaling regulates molar tooth size and cusp development (Sarkar and Sharpe, 2000). Consistent with this, forced inducible *in vivo* expression of *Dkk1* in oral and dental epithelia from the cap stage results in formation of blunted molar cusps, down-regulation of the enamel knot marker *p21*, and expanded expression of the Sclerostin-domain-containing-1 gene (*Sostdc1*), also known as Uterine Sensitization Associated Gene-1 (*Usag-1*), Ectodin, or Wise, whose expression is normally excluded from the enamel knot. *Sostdc1* modulates the Wnt and BMP pathways in a context-dependent fashion (Itasaki *et al.*, 2003). Consistent with roles for Wnt and BMP signaling in regulating cusp patterning, molar teeth in mouse mutants lacking *Sostdc1* have enlarged enamel knots and extra cusps (Kassai *et al.*, 2005). The results of these experiments reveal requirements for Wnt activity in maintaining secondary enamel knots and indicate that tight regulation of this pathway is essential for controlling the shapes of individual teeth (Liu *et al.*, 2008).

(5) Secretory Stage

Wnt10a is expressed in secretory odontoblasts and co-localizes with dentin sialophosphoprotein (*Dspp*), a tooth-specific non-collagenous matrix protein regulating dentin mineralization.

Analysis of *in vitro* data indicates that *Wnt10a* is an upstream regulatory molecule for *Dspp* expression, and that cell-matrix interaction is essential for the induction of *Dspp* expression (Yamashiro *et al.*, 2007). Overexpression of *Wnt3* in dental epithelium results in the loss of ameloblasts and a reduction of enamel in post-natal incisors, suggesting that inhibition of the Wnt pathway might play a role in enamel formation (Millar *et al.*, 2003). In line with this, *Dkk1* is prominently up-regulated in pre-odontoblasts and secretory odontoblasts, while *Dkk3* is transiently expressed in ameloblasts before enamel matrix secretion (Fjeld *et al.*, 2005).

(6) Post-natal Stages

It is generally accepted that Hertwig's epithelial root sheath (HERS) is important for tooth root formation. Syndecan-1, a cell-surface heparan sulfate proteoglycan that enhances Wnt/ β -catenin signaling (Liu *et al.*, 2003), is expressed in the HERS (Yamamoto *et al.*, 2004). The formation of cementum is sensitive to intra- and extracellular phosphate/pyrophosphate distribution, and treatment of immortalized mouse cementoblasts with inorganic phosphate alters the expression of several Wnt pathway genes: Expression of the secreted canonical Wnt signaling *Sfrp4* inhibitor is enhanced, while expression of the *Wif1* and *Dkk3* inhibitors and the *Wnt10b* and *Wnt4* ligands is diminished (Rutherford *et al.*, 2006). *Sfrp4* has been identified as a potential phosphatonin (Berndt *et al.*, 2003), a putative circulating regulator of phosphate concentration. Analysis of these data suggests that Wnt signaling may be involved in cementum formation.

Unlike molar teeth, rodent incisor teeth erupt continuously, a process thought to depend on a pool of stem cells in the cervical loop. Wnt/ β -catenin pathway activity is detected in mesenchymal and epithelial cells of post-natal incisor teeth (Lohi *et al.*, 2010; Rooker *et al.*, 2010; Suomalainen and Thesleff, 2010). Interestingly, however, pathway activity is excluded from the cervical loop, suggesting that, in contrast to its roles in controlling stem cell function in several other regenerating epithelial tissues, such as the hair follicle and intestine, Wnt/ β -catenin signaling does not directly regulate the proliferation and self-renewal of incisor tooth stem cells (Suomalainen and Thesleff, 2010).

(7) β -catenin Signaling and Patterning of the Dentition

Rodents have a toothless region, known as the diastema, between their incisor and molar regions. However, in many rodents, including mice, rudimentary supernumerary tooth germs are present in the diastema and incisor regions early in development, and regress through apoptosis (Keranen *et al.*, 1999; Murashima-Suginami *et al.*, 2007). In mice lacking *Sostdc1*, in addition to abnormal molar cusp patterning, rudimentary incisors survive and erupt as supernumerary teeth, and ectopic molars develop (Kassai *et al.*, 2005; Murashima-Suginami *et al.*, 2007; Munne *et al.*, 2009). Similar phenotypes are observed in mice hypomorphic for LRP4, a SOSTDC1 receptor (Ohazama *et al.*, 2008). Nuclear β -catenin and BMP signaling pathways are up-regulated in dental mesenchymal cells within

the rudimentary incisors of *Sostdc1* mutant mice, and supernumerary tooth formation can be rescued by application of either the BMP inhibitor Noggin or the Wnt inhibitor DKK1 in organ culture, suggesting that *Sostdc1* suppresses tooth development by inhibiting Wnt and BMP signaling in the dental mesenchyme (Murashima-Suginami *et al.*, 2008; Munne *et al.*, 2009). Consistent with these data, forced activation of β -catenin signaling by mutation of the *Ctnnb1* gene to a constitutively active form in oral epithelial cells, or by deletion of epithelial *Apc*, results in continuous tooth development in embryogenesis (Jarvinen *et al.*, 2006; Kuraguchi *et al.*, 2006; Liu *et al.*, 2008). Strikingly, inducible deletion of epithelial *Apc* or activation of β -catenin can result in new tooth development, even in adult mice. This effect is seen only in the continuously erupting incisor, likely reflecting the continued presence of a dental lamina in these specialized teeth (Wang *et al.*, 2009) (F.L and S.E.M., unpublished observations). Analysis of these data, taken together, identifies Wnt/ β -catenin as a pro-odontogenic signal at tooth initiation and in cusp development, and suggests manipulation of this pathway as an important component in strategies for dental tissue engineering.

WNT/ β -CATENIN SIGNALING IN THE DEVELOPMENT OF TASTE PAPILLAE AND TASTE BUDS

Taste buds (TBs) are found on the dorsal surface of the tongue, the posterior soft palate, and the epiglottis. In the tongue, they are located within the tips of the fungiform papillae and in clusters in the epithelium of vallate and foliate papillae. TBs are derived from surface oral epithelial cells (Barlow and Northcutt, 1995; Stone *et al.*, 1995), and, like other epithelial cells, are regenerated at regular intervals during adult life. Unlike other epithelia, however, taste buds have several characteristics usually associated with neurons, including their expression of chemoreceptors, and their ability to synapse with nerve fibers. Each TB consists of ~50 to 100 cells, and is flask-like in shape, with its broad base resting on the corium, and its neck opening into an orifice, the gustatory pore, between the cells of the epithelium. TBs are composed of basal cells and 3 kinds of elongated cells derived from basal cells, denoted types I, II, and III. The type I cells, also called dark cells, are considered supporting cells. Type II cells, also called light cells, are taste sensory cells that detect sweet, umami, or bitter tastes and express the corresponding G-protein-coupled taste receptors. Most type II cells appear to respond to only one type of taste stimulus (Tomchik *et al.*, 2007). Type III cells, also called intermediate cells, are generally considered to be the only taste cell type that makes conventional synaptic contacts with nerve fibers (Yang *et al.*, 2000), and most of these pre-synaptic cells respond to two or more different taste qualities, including sour and salty stimuli (Tomchik *et al.*, 2007).

The fungiform papillae form in a stereotypical array on the anterior two-thirds of the dorsal lingual surface. In mice, these papillae appear first at embryonic day (E) 12.5 as localized epithelial thickenings, termed 'placodes', that show specifically elevated expression of the signaling molecules *Shh* and *Bmp4*

(Hall *et al.*, 1999; Zhou *et al.*, 2006). By E14.5, fungiform placodes begin to evaginate, forming raised papillae with mesenchymal cores. Taste buds are first morphologically evident at approximately E18 as onion-shaped aggregates of elongated cells within apical papillary epithelium (Stone *et al.*, 1995). However, early in papillary morphogenesis, a cluster of cells at the center of the papillary apex can be distinguished from surrounding cells by virtue of their mitotic quiescence and expression of the taste cell marker cytokeratin 8 (Farbman and Mbiene, 1991; Mbiene and Roberts, 2003; Zhou *et al.*, 2006). Recent evidence suggests that *Shh*-expressing cells in the fungiform placode are determined precursor cells of taste buds, and do not differentiate into surface epithelial cells (Thirumangalathu *et al.*, 2009). These results indicate that taste bud progenitor cells are established early in papillary development.

Several Wnt pathway genes, including *Wnt6*, *Wnt10a*, *Wnt10b*, *Fzd6*, β -catenin (*Cttnb1*), and *Lef1*, are expressed in developing fungiform placodes, and Wnt reporter transgenes detect Wnt/ β -catenin signaling pathway activity in developing taste papillae and taste buds. Forced epithelial expression of the secreted Wnt inhibitor DKK1, or genetic deletion of epithelial β -catenin, down-regulates expression of *Shh* and *Bmp4*, two early markers of taste organ development, and blocks initiation of the development of fungiform papillae and taste buds (Liu *et al.*, 2007). These data indicate that Wnt signaling is required within epithelial cells for fungiform placode formation. Similar, though less severe, phenotypes are detected in embryos lacking either *Lef1* or *Wnt10b* (Iwatsuki *et al.*, 2007), indicating that these Wnt pathway components play key roles in taste development, but may overlap in their functions with other LEF/TCF family members and Wnt ligands, respectively. Conversely, forced activation of epithelial Wnt signaling *in vivo* up-regulates expression of *Shh*, and produces numerous massively enlarged ectopic fungiform papillae and taste buds in the tongue, while filiform papilla morphogenesis is almost absent, indicating that early up-regulation of Wnt signaling promotes development of fungiform taste papilla and taste bud development at the expense of filiform papillae (Liu *et al.*, 2007). Up-regulation of *Shh* by Wnt signaling has been confirmed by *in vitro* culture of embryonic mouse tongues, while blocking *Shh* signaling *in vitro* enhances papilla formation and is accompanied by an up-regulation of Wnt/ β -catenin signaling, indicating that *Shh* limits Wnt/ β -catenin pathway activity in this system (Iwatsuki *et al.*, 2007). Another downstream target of Wnt/ β -catenin in papillary development is the SoxB1-HMG-box transcription factor gene *Sox2*. *Sox2* is expressed in basal epithelial cells of the tongue, with high levels in taste bud placodes, fungiform papillae, and mature taste cells, and low levels in filiform papillae. In hypomorphic *Sox2* embryos whose *Sox2* expression is 20% of the normal level, fungiform placodes form, but no mature taste buds develop. Conversely, transgenic overexpression of *Sox2* in basal cells inhibits differentiation of filiform keratinocytes, suggesting that *Sox2* functions in a dose-dependent manner to promote the differentiation of tongue progenitor cells into taste bud sensory cells rather than keratinocytes (Okubo *et al.*, 2006). Deletion of the TGF β antagonist follistatin, which is expressed in tongue mesenchyme, causes increased expression of mesenchymal BMP7 and altered patterning of epithelial Wnt/ β -catenin

activation and papilla formation, indicating important roles for mesenchymal-epithelial signaling in controlling Wnt pathway activity and papillary development (Beites *et al.*, 2009).

In vitro experiments have shown that BMP and SHH signaling pathways regulate the formation of taste papillae (Hall *et al.*, 2003; Mistretta *et al.*, 2003; Zhou *et al.*, 2006). However, the *in vivo* roles of these pathways in taste organ development, and *in vivo* interactions among Wnt, BMP, and SHH signaling pathways, have not yet been fully elucidated. Another key question for future research is how activation of the Wnt pathway at similar embryonic stages in different oral epithelia leads to such different outcomes, namely, formation of teeth in the jaws, and taste papillae in the tongue. In each case, activation of β -catenin signaling provides the instruction "make an appendage", but the nature of the appendage formed seems to rely on pre-existing regional signals. Possible mechanisms underlying these regional differences in response to Wnt activation are discussed in more detail below. Another critical question for oral-regenerative medicine is whether Wnt, BMP, and SHH pathways are involved in the cyclical regeneration of taste buds that occurs in adult life and is essential for homeostatic maintenance of taste function, and for recovery from taste cell injury such as can occur following chemotherapy. Periodic growth of hair follicles, another type of ectodermal appendage, is known to be regulated by Wnt, SHH, and BMP signaling (Millar, 2002; Fuchs, 2008). It will be fascinating to determine whether similar principles apply to the taste bud.

WNT/ β -CATENIN SIGNALING IN DEVELOPMENT OF THE LIP AND PALATE

Cleft lip with or without cleft palate (CLP) has an occurrence of 1 in 500 to 1 in 2500 live births worldwide, making it the most common craniofacial birth defect in humans (Vanderas, 1987; Schutte and Murray, 1999). In embryogenesis, the vertebrate upper lip forms from initially freely projecting maxillary, medial nasal, and lateral nasal prominences at the rostral and lateral boundaries of the primitive oral cavity, that subsequently undergo fusion to form a mature lip (Jiang *et al.*, 2006). Mutations in the *WNT3* and *WNT9B* genes are associated with CLP in both humans and mice, indicating that Wnt signaling plays a critical role in this process.

In a mouse model of human CLP, the A/WySn strain, an essential causal recessive mutation, *clf1*, was mapped to a small region of mouse chromosome 11 containing the closely linked *Wnt3* and *Wnt9b* genes (Juriloff *et al.*, 2001). Sequence analysis showed that *clf1* is associated with a retrotransposon insertion at 6.6 kb downstream of the *Wnt9b* gene, and non-complementation of *clf1* and a *Wnt9b* null mutation in a standard genetic test of allelism confirmed that *clf1* affects the *Wnt9b* gene (Juriloff *et al.*, 2006). In line with this, a targeted mutation in the mouse *Wnt9b* gene caused an incomplete penetrance of CLP in homozygous mutants (Carroll *et al.*, 2005).

The *clf1* region of mouse chromosome 11 is syntenic to human Chromosome 17q21, a chromosomal region strongly associated with non-syndromic CLP in some human populations (Chenevix-Trench *et al.*, 1992; Shaw *et al.*, 1993; Mitchell, 1994; Peanchitlertkajorn *et al.*, 2003; Marazita *et al.*, 2004;

Moreno *et al.*, 2004). In humans, a nonsense mutation in the *WNT3* gene is associated with tetra-amelia, a rare recessive genetic disorder characterized by complete absence of all 4 limbs and other anomalies, including CLP (Niemann *et al.*, 2004). In addition, non-syndromic cleft lip with or without cleft palate (NSCLP) is associated with chromosome 3p21.2, which contains the *WNT5A* gene (Blanton *et al.*, 2004).

Both *Wnt3* and *Wnt9b* are expressed in the facial ectoderm at critical stages of midfacial morphogenesis during mouse embryogenesis (Lan *et al.*, 2006), with *Wnt3* expression in the maxillary and medial nasal ectoderm, and *Wnt9b* expression in the maxillary, medial nasal, and lateral nasal ectoderm. During mouse lip fusion, *Wnt9b*, but not *Wnt3*, is expressed in the epithelial seam between the fusing medial and lateral nasal processes, and the Wnt/ β -catenin reporter transgene *TOPGAL* is specifically activated in distal regions of the medial nasal, lateral nasal, and maxillary processes prior to lip fusion. During lip fusion, the epithelial seam between the medial and lateral nasal processes, and facial mesenchyme directly beneath the fusing epithelia, express *TOPGAL*. Together with *Wnt3* and *Wnt9b* mutant phenotypes, analysis of these data suggests that *Wnt3* and *Wnt9b* signal through the Wnt/ β -catenin pathway to regulate lip fusion (Lan *et al.*, 2006). Interestingly, deletion of R-spondin2, another secreted protein capable of activating the Wnt pathway, also results in craniofacial malformations in mice (Yamada *et al.*, 2009). Combined mutation in mouse embryos of the downstream Wnt pathway components *Lef1* and *Tcf4* results in a more "human-like" appearance of the fetal head, indicating a critical role for Wnt signaling in the growth of the maxillary and frontonasal prominences. This finding has led to the suggestion that subtle differences in the timing or duration of Wnt signaling may underpin species-specific differences in facial growth (Liu *et al.*, 2009).

TISSUE-SPECIFIC ROLES OF WNT/ β -CATENIN SIGNALING IN THE DEVELOPMENT OF ORAL TISSUES

As discussed above, initiation of the development of oral ectodermal appendage organs, such as taste buds and teeth, is dependent on activity of the Wnt/ β -catenin pathway, and can be promoted by forced activation of β -catenin signaling (Jarvinen *et al.*, 2006; Liu *et al.*, 2007, 2008; Wang *et al.*, 2009). How does activation of the same signaling pathway have such diverse effects on the oral ectoderm, promoting tooth development in some locations, and taste bud formation in others, as well as being required in a host of other critical functions, including skeletal development and lip and palate fusion? The answer to this question is likely to lie, at least in part, in a thorough understanding of the biological contexts in which various craniofacial cells find themselves at different developmental time-points and in different spatial locations. In other words, the precise response to the activation of Wnt signaling must depend on combinations of interacting signaling pathways, transcription factors and co-factors, and other intracellular and extracellular modulators of pathway activity that are expressed in, or received by, the cell at the time of Wnt ligand reception. The site-specific differentiation

of oral tissues is likely determined by signals from the mesenchyme, as indicated by the ability of dental mesenchyme to reprogram non-dental epithelia to a dental fate in tissue-recombination experiments (Mucchielli *et al.*, 1997). The mesenchymal signals may be determined by the expression patterns of HOX genes, a family of homeodomain transcription factors that act to specify positional identity during development, similar to a mechanism that has been described to control regional development of the epidermis (Rinn *et al.*, 2006). TCF/LEF transcription factors exist as multiple isoforms that display distinct expression patterns and co-factor binding properties (Hoppler and Kavanagh, 2007). TCF/LEF isoform diversity likely contributes significantly to context-specific Wnt activity. For example, in *Xenopus* development, Tcf1 and Tcf3 mediate Wnt signaling in mesoderm induction, while Wnt-mediated regulation of ventrolateral patterning, which occurs slightly later in development, requires Tcf1 and Lef1 (Liu *et al.*, 2005). In mouse embryos, both Lef1 and Tcf1 are expressed in tooth buds, while Tcf11 is exclusively expressed in the palate and Lef1 is exclusively expressed in the tongue and lips (Oosterwegel *et al.*, 1993; van Genderen *et al.*, 1994). These observations suggest that different utilization of Tcf/Lef factors may contribute to the specificity of Wnt signaling in the development of these distinct oral tissues. Analysis of emerging data suggests that cell-type-specific chromatin remodeling complexes also play key roles in mediating tissue-specific transcriptional responses to activation of generally utilized signaling pathways (Lange *et al.*, 2008). While we are beginning to understand some of these interactions, gaining a full picture of the signaling, transcriptional, and epigenetic regulators of these complex processes will likely require computer simulation and mathematical modeling approaches in addition to genetic, genomic, developmental, molecular, and bioinformatics studies.

THE WNT/ β -CATENIN PATHWAY IN ORAL DISEASE

(1) Inherited Diseases

In addition to the association of *WNT3* mutations with cleft lip and palate, as described above, several other congenital human syndromes are associated with mutations in Wnt pathway genes (Table 1). Nonsense, missense, and translation termination mutations in the *WNT10A* gene are associated with ectodermal dysplasias, including odonto-onycho-dermal dysplasia, a rare autosomal-recessive syndrome in which patients display dry hair, severe hypodontia, smooth tongue with marked reduction of fungiform and filiform papillae, onychodysplasia, keratoderma and hyperhidrosis of palms and soles, and hyperkeratosis of the skin (Adaimy *et al.*, 2007; Bohring *et al.*, 2009; Nawaz *et al.*, 2009). These characteristics are reminiscent of phenotypes caused by inhibiting Wnt/ β -catenin signaling in mouse embryonic ectoderm (Kratochwil *et al.*, 1996; Jarvinen *et al.*, 2006; Iwatsuki *et al.*, 2007; Liu *et al.*, 2007, 2008), suggesting that *WNT10A* activates the β -catenin pathway in the development of these epithelial organs.

Loss-of-function mutations in the *APC* gene, the activity of which normally limits Wnt pathway activity, cause oral and maxillofacial symptoms of familial adenomatous polyposis (FAP), including an increased risk of jaw osteomas, odontomas,

and supernumerary or unerupted teeth (Gardner's syndrome), in addition to colorectal cancer (Foulkes, 1995). By contrast, non-sense or frameshift loss-of-function mutations in the intracellular Wnt pathway inhibitor *AXIN2* are associated with dominant inherited severe permanent tooth agenesis (oligodontia) and colorectal neoplasia (Lammi *et al.*, 2004). In addition, *AXIN2* polymorphisms are reported as a risk factor for selective tooth agenesis, the most common developmental abnormality of the human dentition (Mostowska *et al.*, 2006). Tooth agenesis in these conditions is likely the result of continuous Wnt pathway stimulation in the absence of *AXIN2*, resulting in disrupted dental morphogenesis and development of ectopic, abnormal teeth that do not erupt. A distinct form of inherited agenesis of permanent teeth, He-Zhao deficiency, was discovered in a Chinese kindred, in which the affected locus was mapped to chromosome 10q11.2 (Liu *et al.*, 2001), close to the gene encoding the secreted Wnt inhibitor *DKK1* (Roessler *et al.*, 2000). Consistent with results from mouse models, analysis of these data indicates that inappropriate activation of Wnt signaling can lead to either supernumerary teeth or tooth agenesis, and indicates that tight control of Wnt pathway activity is necessary for normal tooth development. Importantly, a diagnosis of supernumerary teeth or congenital tooth agenesis may be considered a possible indicator of colorectal cancer susceptibility.

Other craniofacial structures, in addition to the lip, palate, teeth, hair follicles, and taste papillae, can also be affected by Wnt pathway mutations. For instance, gain-of-function mutations in the gene encoding *LRP5* causes high bone mass. A 59-year-old woman carrying a novel *LRP5* missense mutation, Arg154Met, manifested extensive oropharyngeal exostoses as well as dense bones, showing that exuberant *LRP5* effects are not always benign (Rickels *et al.*, 2005).

(2) Tumors

Ectopic activation of the Wnt signaling pathway is associated with many human tumors, including those derived from oral tissues (Table 1). Nuclear localized β -catenin is observed in follicular and plexiform-type ameloblastomas, and these tumors are occasionally associated with gain-of-function mutation of β -catenin or loss-of-function mutation of *APC* (Kawabata *et al.*, 2005; Miyake *et al.*, 2006; Siriwardena *et al.*, 2009). Craniopharyngioma is a rare neoplasm that occurs in the sellar region. No β -catenin mutation is present in any papillary craniopharyngiomas analyzed. By contrast, gain-of-function β -catenin gene mutations are present in all adamantinomatous craniopharyngiomas examined; these lesions have a histological appearance similar to that of ameloblastoma (Sekine *et al.*, 2002). Calcifying odontogenic cyst (COC), a rare odontogenic lesion that displays histologic features such as ghost cell formation, a predominantly cystic morphology, and calcification, is also frequently associated with gain-of-function mutation of β -catenin (Sekine *et al.*, 2003). These data indicate that Wnt/ β -catenin signaling is important in the formation of certain oral tissue tumors, as well as in embryonic development of the various structures of the oral cavity.

Although little is known about the role of Wnt signaling in development of the salivary gland, the proto-oncogenic effects of Wnt activation in mouse salivary gland tissue have been well-established by studying mutant *Apc* allele Multiple intestinal neoplasia (*Min/+*);*Tcf1*^{-/-} mice (Roose *et al.*, 1999), *MMTV-Wnt1* transgenic mice (Tsukamoto *et al.*, 1988; Li *et al.*, 2001), and mice with *MMTV-Cre*-mediated forced activation of Wnt/ β -catenin signaling (Bierie *et al.*, 2003). Among human pleomorphic adenomas, the most common tumors of the salivary gland, chromosome 12q13-15 re-arrangements involving the oncogene *HMG2* gene are frequent. In some cases, these re-arrangements cause production of a fusion protein containing *HMG2* and WNT inhibitory factor 1 (*WIF1*), resulting in increased *HMG2* and decreased *WIF1* expression in the salivary tissue, and tumors possibly arising due to synergistic effects of increased Wnt and *HMG2* activity (Queimado *et al.*, 2007). Wnt pathway components are overexpressed in adenoid cystic carcinoma (ACC), another common salivary gland tumor subtype, and nuclear localized β -catenin and mutations in Wnt pathway genes, including β -catenin, *AXIN1*, and *APC*, are detected in some cases (Frierson *et al.*, 2002; Daa *et al.*, 2004, 2005). However, in an immunohistochemical study of biopsy specimens including 12 pleomorphic adenoma, 17 ACC, 10 epithelial-myoepithelial carcinomas, and 4 polymorphous low-grade adenocarcinomas, nuclear staining of β -catenin was observed only in the epithelial-myoepithelial carcinomas, and not in other salivary tumors (Furuse *et al.*, 2006), indicating the divergence in tumorigenesis of salivary gland tumors, and lack of consensus on the probable role of β -catenin signaling in various subtypes.

Squamous cell carcinoma of the head and neck (HNSCC) represents a large health burden worldwide, with approximately 500,000 cases diagnosed annually (Kim *et al.*, 2001). The oral cavity is a major site of origin for HNSCC; for instance, in the year 2000, 30,200 cases of oral cavity and pharynx cancer were diagnosed and were responsible for an estimated 7800 deaths in the United States (Greenlee *et al.*, 2000). Expression analyses of HNSCC and HNSCC-derived cell lines have found that most tumors and cell lines examined overexpress Wnt signaling pathway components (Leethanakul *et al.*, 2000; Uraguchi *et al.*, 2004), and that nuclear β -catenin localizes at the invasive front in carcinomas (Uraguchi *et al.*, 2004). Treatment of HNSCC cells with anti-WNT1 antibody inhibits proliferation and induces apoptosis (Rhee *et al.*, 2002), while enhancing Wnt/ β -catenin signaling inhibits apoptosis, induces cell scattering and invasive growth in matrigel, and promotes HNSCC tumor growth in nude mice (Yang *et al.*, 2006).

In oral squamous cell carcinoma (OSCC), cytoplasmic accumulation of β -catenin is a common characteristic, but is not closely associated with mutational alterations in the *APC*, β -catenin, and *AXIN1* genes (Iwai *et al.*, 2005; Odajima *et al.*, 2005). Analysis of these data suggests that Wnt/ β -catenin signaling may play a role in oral squamous cell carcinoma, but that this aberrant signaling may be secondary or due to epigenetic changes. Interestingly, epigenetic inactivation of *SFRP* genes, that encode secreted WNT inhibitors, is frequently detected in OSCC (Sogabe *et al.*, 2008).

Table 1. Human Oral Diseases with Aberrant Wnt/ β -catenin Signaling Activity

Disease	Abnormality of Oral Tissue	Mutated Gene	Effect on Canonical		Reference
			Wnt Signaling		
Tetra-amelia	Cleft lip	<i>WNT3</i>	Down		Niemann <i>et al.</i> , 2004
Odonto-onycho-dermal dysplasia	Severe hypodontia, smooth tongue	<i>WNT10A</i>	Down		Adaimy <i>et al.</i> , 2007; Bohring <i>et al.</i> , 2009; Nawaz <i>et al.</i> , 2009
Gardener's syndrome	Jaw osteomas, odontomas, and supernumerary or unerupted teeth	<i>APC</i>	Up		Foulkes, 1995
Oligodontia	Severe permanent tooth agenesis	<i>AXIN2</i>	Up		Lammi <i>et al.</i> , 2004
COC	Calcifying cystic odontogenic tumor	<i>CTNNB1</i>	Up		Sekine <i>et al.</i> , 2003
Adenoid cystic carcinoma	Salivary gland tumor	<i>CTNNB1, APC, AXIN1</i>	Up		Daa <i>et al.</i> , 2004
Pleomorphic adenomas	Salivary gland tumor	<i>WIF</i>	Up		Queimado <i>et al.</i> , 2007

In oral leukoplakia, a precancerous lesion that sometimes develops into squamous cell carcinoma, *WNT3* expression, and nuclear β -catenin are detected in individuals with dysplasia, but not in normal oral epithelium, suggesting potential involvement of this pathway in dysplasia (Ishida *et al.*, 2007). This oral pre-malignant lesion is the subject of study in many primary prevention trials, and small-molecule Wnt signaling antagonists may be promising candidates for such purposes.

FUTURE DIRECTIONS

The advances outlined here underscore the broad utilization of Wnt/ β -catenin signaling in the development of a diverse array of oral tissues. While research over the past several decades has revealed many of the functions of this pathway in the oral cavity, some areas have received little attention to date. The role of Wnt/ β -catenin signaling in salivary gland morphogenesis and regeneration is one such area. Delineating the role of Wnt signaling in salivary gland biology is likely to be of value in improving our understanding of the molecular mechanisms at work in salivary cancers, as well as in normal salivary development.

Current findings raise several questions and opportunities related to clinical applications. A major question is whether we can utilize our knowledge of the molecular controls of taste, tooth, and other oral tissue development in bioengineering approaches to generate new organs such as teeth, in promoting taste bud regeneration following chemotherapy, or in therapeutic contexts—for instance, in repairing cleft lip and palate. Based on the known effects of forced activation of Wnt/ β -catenin signaling in oral tissues, small-molecule agonists of this pathway could potentially be useful in such applications. For instance, one such agonist, IQ-1, has been shown to be capable of maintaining long-term expansion of mouse embryonic stem cells and preventing spontaneous differentiation (Miyabayashi *et al.*, 2007). As discussed above, numerous lines of evidence suggest that inappropriate activation of Wnt/ β -catenin signaling underlies the etiology of at least some oral cancers. Therefore, development of any such therapeutic applications must include careful regulation of dose, and methods for temporal and spatial control of pathway activation. In addition, a better understanding of the biological context

required for specific organ development and/or regeneration in response to activation of Wnt signaling, as discussed above, will facilitate the development of novel therapeutic approaches.

A second avenue of interest lies, conversely, in the development of methods for dampening Wnt/ β -catenin signaling activity as a contributing therapy in oral cancer treatment. A promising approach in this area is the use of small-molecule Wnt pathway inhibitors. For instance, nitric-oxide-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs), which have pleiotropic effects, including the inhibition of Wnt/ β -catenin signaling, are emerging as a promising class of compounds for the chemoprevention of colon cancer (Rigas, 2007). Progress is also being made in the development of specific Wnt/ β -catenin small-molecule antagonists. Some examples include FJ9, a small molecule that targets interaction of Fzd and Dsh, induces apoptosis in human lung cancer and melanoma cells, and suppresses tumor cell growth in a mouse xenograft model (Fujii *et al.*, 2007). Another small-molecule antagonist, PKF115-584, which disrupts transcriptionally active β -catenin/TCF protein complexes, blocks expression of Wnt target genes and induces cytotoxicity in multiple myeloma cells from both patients and cell lines without a significant effect in normal plasma cells (Sukhdeo *et al.*, 2007). Some more recently discovered examples include small molecules that abrogate destruction of Axin proteins or inhibit the activity of Porcupine, a membrane-bound acyltransferase essential for Wnt protein production (B Chen *et al.*, 2009; Huang *et al.*, 2009). A more thorough understanding of the precise contributions of Wnt pathway activation to the development of oral cancers will clearly be a prerequisite for the development of successful therapies for these classes of disease. If aberrant Wnt/ β -catenin signaling is confirmed as an underlying mechanism in certain oral cancers, small-molecule Wnt pathway antagonists may hold promise in the prevention and treatment of oral tumors.

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