Review Article

WNT Signaling and Implications for Dental Regenerative Medicine

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Abstract

WNT signaling pathway functions throughout the lifetime of the organism by ensuring the normal patterning of tissues during development and by regulating self-renewal and cell fate commitment in multiple adult stem cell types. Currently, not enough is known about the role of WNT signaling in the maintenance of adult dental tissues. In this review we briefly outline current developments in the field of WNT signaling with regard to the regulation of mesenchymal dental pulp cells and pose outstanding questions to be addressed in the future.

ABBREVIATIONS

ESC: Embryonic Stem Cells; FZD: Frizzled Receptors; APC: Adenomatous Polyposis Coli; CKIα: Casein Kinase I Alpha; GSK3: Glycogen Synthase Kinase 3; DSPP: Dentin Sialophosphoprotein; SNP: Single Nucleotide Polymorphism; DPSCS: Dental Pulp Stem Cells

INTRODUCTION

A new paradigm has emerged, suggesting that in cycling tissues, adult stem cells achieve a fine balance between self-renewal and differentiation as a result of stochastic cell fate decisions involving either a cell-autonomous or cell-extrinsic mode of action [1,2]. Central to understanding the mechanisms of stem cell behavior is the need to further investigate the ability of different niche growth factors to communicate context-dependent developmental cues to stem cells in the niche. A highly conserved pathway that has been implicated in the self-renewal and differentiation of embryonic (ESC) and adult stem cells is the WNT/ β-catenin signaling pathway. WNT ligands are glycoprotein molecules that have a short-range mode of action due to lipid modifications, which render them hydrophobic [3] and tether them to their cognate Frizzled (FZD) receptors and LRP5/6 family co-receptors on cell membranes [1]. Thus, by being highly insoluble, WNT ligands can only exert an effect on neighboring cells within the limited range of the niche [1].

In the light of recent advances in the field and consistent with studies implicating WNT pathway components in the functioning of the mitotic spindle during division, mitosis and cell fate determination appear to converge at the level of WNT signaling [4,5]. Following the purification of an active WNT3a ligand, a well-known canonical WNT ligand, which has previously been shown to maintain mouse ESCs in a self-renewing state [6] and to drive mesodermal and endodermal differentiation in human ESC [7], it has been eloquently demonstrated that a targeted delivery of WNT3a can induce asymmetric inheritance of cell fate determinants in mouse ESCs [8]. The case for WNT signaling in the maintenance of the adult stem cell pool during homeostasis is further reinforced as aberrant WNT signaling often underlies chromosome instability and tumor progression in tissues that exhibit high rates of cellular turnover [9].

WNT signaling can be classified into canonical and non-canonical signaling. Non-canonical signaling pathways, which have been known to regulate cell polarity and cell movement, act independently of β-catenin [10], which is a key component of the canonical WNT pathway.

In the absence of a WNT signal, β-catenin binds to a multimeric destruction complex composed of APC (adenomatous polyposis coli), AXIN (1/2), casein kinase I alpha (CKIα) and glycogen synthase kinase 3 (GSK3), where it is phosphorylated by CKIα and GSK3 and targeted for proteasome degradation [1] (Figure 1). When present, WNT ligands engage with Frizzled receptors and LRP5/6 co-receptors, ultimately leading to clustering of these receptors in complexes. The conformational changes ensuing this coupling enable the LRP cytoplasmic tail to be phosphorylated by GSK3 and CKIα, resulting in the sequestration of the scaffold protein AXIN to the membrane and the inhibition of GSK3 [1,11]. Stabilized β-catenin then translocates to the nucleus and initiates Lef/Tcf - dependent transcription of global and tissue-specific WNT target genes. Lineage tracing experiments using broadly activated WNT target genes such as AXIN2 and LGR5,
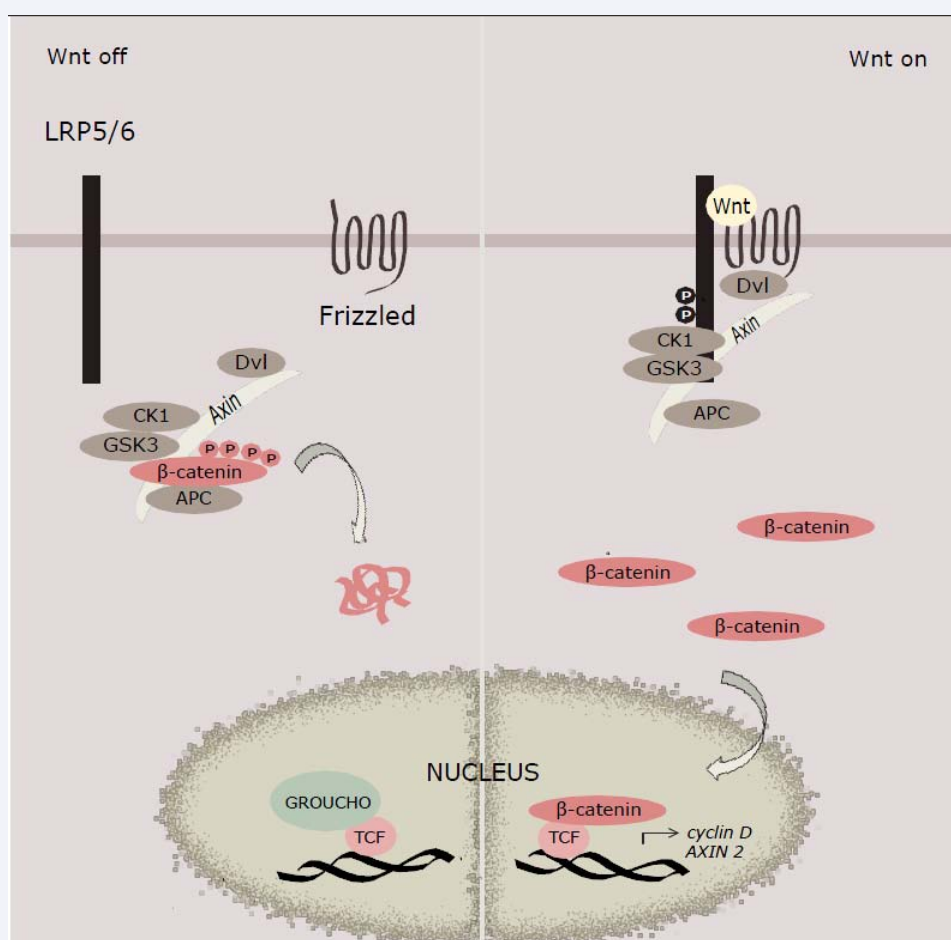
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Figure 1 Overview of the canonical WNT signaling pathway. In the absence of a WNT signal β-catenin binds to a destruction complex composed of AXIN, GSK3, CK1 and APC where it is phosphorylated by CK1 and GSK3 and is continuously targeted for proteosomal degradation. When a WNT signal engages with the Frizzled receptor, this leads to association of AXIN with phosphorylated LRP, whereby GSK3 is inhibited leading to stabilization of β-catenin levels in the cytoplasm. β-catenin then translocates to the nucleus, where it outcompetes the transcriptional co-repressor GROUCHO for TCF binding and initiates WNT target gene transcription.

which encode a WNT negative regulator and novel type of WNT receptor, respectively, have facilitated the identification of stem cell populations in tissues of the intestine, the skin the hair follicle and the mammary gland [12-15] (Table 1).

WNT SIGNALING − ROLE IN TOOTH DEVELOPMENT AND DENTAL PULP HOMEOSTASIS

WNT/β-catenin has been strongly implicated in the establishment of craniofacial structures during embryogenesis and is known to govern multiple stages of dental morphogenesis [reviewed in 16]. Expression of only two WNT ligands has been confirmed in pulp mesenchymal cells and/or odontoblasts. WNT11, which is expressed in rat dental pulp, has been shown to positively regulate odontoblast marker expression in mouse dental papilla cells [17]. WNT10A, which is expressed in early odontoblast lineages in the embryo as well as in terminally differentiated secretory odontoblasts in mice, was shown to function upstream of odontoblast differentiation marker DSPP (dentin sialophosphoprotein) [18]. Early evidence in support of the role of WNT signaling in normal tooth development was presented by Lammi *et al.* [19] who were able to show that a

functional AXIN2 protein is indispensable for the formation of teeth. Interestingly, mutations in the *Axin2* gene, which are associated with familial tooth agenesis (oligodontia), have also been linked to a higher risk of developing colon cancer. SNP and haplotype analyses have revealed a significant correlation between *WNT3* polymorphisms and the development of non-syndromic cleft lip with or without cleft palate [20]. A strong association has also been uncovered between polymorphic variants of the *WNT10A* gene and non-syndromic tooth agenesis in the Polish population [21]. The identification of mutations in the coding region of *WNT10A* has further corroborated the involvement of the *WNT10A* gene in non-syndromic tooth agenesis [21].

Accumulating evidence suggests that WNT signaling is involved in the regulation of mesenchymal dental pulp cells. Further to identifying a WNT responsive population in the dental pulp of mice, Hunter *et al.* [22] have demonstrated that augmentation in endogenous WNT signaling is associated with enhanced cell proliferation of human dental pulp stem cells (DPSCs). In an attempt to mimic the enhancement of WNT signaling which occurs in tissues at sites of injury *in vivo*, it

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Wnt-associated proteins	Description
APC — adenomatous polyposis coli	organizes β-catenin destruction complex
GSK3 — glycogen synthase kinase 3	β-catenin phosphorylation
CK1α — casein kinase 1 α	β-catenin phosphorylation
CK1γ — casein kinase 1 γ	LRP phosphorylation
AXIN 1	organizes β-catenin destruction complex; binds LRP following pathway activation
AXIN 2	organizes β-catenin destruction complex; cannot bind LRP following pathway activation
DVL — dishevelled	interacts with Frizzled following pathway activation
FZD — Frizzled	Wnt receptor
LRP 5/6 — lipoprotein receptor-related protein	Wnt co-receptor
LRG5 — leucine-rich repeat-containing G-protein coupled receptor 5	R-spondin family ligand receptors; potentiation of Wnt signaling
TCF — T-cell factor	transcription factor; binds β-catenin
Groucho	transcriptional co-repressor; binds TCF
WNT3a	Wnt family ligand
WNT10a	Wnt family ligand
WNT11	Wnt family ligand

Table 1 WNT-related proteins.

was shown that mutant mice (Axin2$^{LacZ/LacZ}$), lacking the critical WNT negative regulator AXIN2, exhibit improved pulp vitality and dentin regeneration in response to acute pulp injury. Similarly, odontoblast differentiation was upregulated following treatment of pulp-injured rats with a liposomal WNT3a protein (L-WNT3a). This means that WNT signaling may provide important cell survival cues to the mesenchymal pulp stem cells, by instructing them to differentiate into dentin producing odontoblasts, thereby enabling the protection of the pulp cavity [22]. Other line of evidence, however, states that signaling through the canonical WNT1 ligand can impede odontoblastic differentiation in human dental pulp stem cells, which might have implications for the self- renewal of human DPSCs [23]. These data are consistent with previous reports suggesting a role for the canonical ligands WNT1 and WNT3a in the suppression of osteogenic differentiation in mesenchymal stem cells [24-26]. Although conflicting reports exist, it is generally believed that spatiotemporally controlled WNT/ β-catenin signaling directs the specification of the dental mesenchyme into the odontoblast and cementoblast lineages during development [27,28]. Examination of the functions of WNT/ β-catenin signaling in adult mice has revealed that all mineralizing tissues of the craniofacial complex (i.e. odontoblasts, osteoblasts, ameloblasts, cementoblasts and mesenchymal pulp cells) sustain WNT responsiveness during homeostasis in the adult. Conditional mouse *Wls* (a chaperone protein that regulates WNTs secretion) knock-outs in osteocalcin - expressing mineralized tissues, have furthermore indicated that unlike in osteoblasts, where osteogenesis is dependent on WNT-driven Runx2 expression for differentiation, in odontoblasts WNT- mediated Runx2 activation is required for the inhibition of odontoblast differentiation [29]. Another report based on similar experimental approaches indicates that abrogated WNT signaling disrupts the normal homeostasis of the periodontal ligament [30]. Needless to say, the cellular response to the same WNT ligand is going to vary between cell types and species. The inability to ascribe universal properties to the same canonical and non-canonical WNTs might be due to context-specific roles in WNT pathway activation, likely explained by a differential genetics background of the WNT-responsive cells and WNT inhibitor availability in the extracellular space across various tissues [31].

CONCLUDING REMARKS

Abundant data suggests that WNT mechanisms regulate dental pulp cell fate choices during development and homeostasis. A more in-depth investigation of the specific mode of action of different WNT ligands on human DSPC will aid the development of protocols for their *in vitro* expansion. In particular, further elucidation of the role of WNT3a in regulation of DPSCs, holds great promise for the treatment of common dental conditions such as dental caries.

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