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Wnt8b: a suppressor of early eye and retinal progenitor formation

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1. Structure

Mouse *Wnt8b* gene (also known as wingless related MMTV integration site 8b, NM_011720) is a member of the *Wnt* gene superfamily that encodes a group of evolutionarily conserved, secreted, cysteine-rich glycoproteins, with 19 Wnt-encoding genes found in the mouse genome. *Xwnt8*, the first member of *Wnt8* subfamily including *Wnt8a*, *Wnt8b* and *Wnt8c*, was originally isolated in *Xenopus* by screening a neurula stage library and is defined by its sequence similarity to *int-1* (mouse) and *wingless* (*Drosophila*), the original founders of the *Wnt* gene superfamily. The primary structure of mouse Wnt-8b protein (Q9WUD6) is comprised of 350 amino acid residues and is characterized by a predicted signal peptide, a conserved 4-element Wnt-8 protein motif and several other motifs (Fig. 1A). The 3-D structure of Wnt-8b as well as other Wnts is unknown mostly due to their protein insolubility.

2. Function

Wnt signaling plays critical roles in the process of cell fate decisions, patterning, morphogenesis, proliferation, tumorigenesis and diseases. Wnt proteins are the ligands that bind cell surface receptors of the Frizzled family to trigger several intracellular pathways. In the canonical Wnt/ β -catenin pathway, binding of Wnt proteins to a receptor complex of Frizzled (Fz) and low-density lipoprotein receptor-related proteins (LRP) causes cytoplasmic accumulation of β -catenin and its subsequent translocation to nucleus where, together with lymphoid enhancer binding factor 1 (LEF1) or T cell specific transcription factor (TCF), it modulates target gene expression including *Axin2*, *cyclin D1* and *MYC* (Fig. 1B). Wnts can also activate noncanonical signaling cascades that involves calcium signaling, small GTPases of the Rho family, and JNK signaling.

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Early eye and forebrain development in vertebrates are closely related. The eye/retina is derived from a single domain (eye field) marked by the expression of transcription factors Rax, Six3, Pax6, Lhx2 and Six6 within the anterior neural plate (ANP), while the forebrain (telencephalon and diencephalons) is regionalized from other domains of ANP. Morphogenetic movement of the eye field results in the sequential formation of symmetric optic pits, optic vesicles and double-layer optic cups. The eye field and optic vesicles are composed of common retinal precursors that give rise to retinal progenitor cells (RPCs) and the cells forming retina pigment epithelium (RPE) that are located at the inner layer and outer layer of optic cup, respectively.

Wnt8b is a suppressor of early eye and RPC formation in vertebrates. In mouse, Wnt8b expression is initiated at E8.5 in the prospective dorsal forebrain (Figs 1C,E). This Wnt8b-positive domain does not protrude into the Six3 expression domain (Figs 1D,F) that marks the prospective rostro-ventral forebrain and optic vesicles. At this stage, Wnt8b expression domain is adjacent to the dorsal optic vesicles that give rise to RPE (Figs 1E,F). Maintaining a Wnt8b-free territory at E8.5 in the rostro-ventral forebrain and eye field is essential for RPC specification, since ectopic Wnt8b expression in this region in transgenic mouse embryos ablated RPC formation, and the cells forming optic vesicles were specified as prospective RPE (Liu et al., 2010). Molecular studies showed that Wnt8b is repressed at the transcriptional level by Six3 in mouse (Fig. 1G) (Liu et al., 2010). Consistent with this observation, gain-of-function of β -catenin activity in mouse eye primordia through genetic deletion of its phosphorylation site interrupted lens morphogenesis and neuroretina development, but did not affect RPE differentiation (Machon et al., 2010). The idea that Wnt8b serves as a negative regulator of early eye development is further supported by zebrafish studies. Wnt8b/ β -catenin signaling suppressed zebrafish early eye formation in a temporally dependent manner. In addition, antagonism of Wnt8b/ β -catenin signaling exerted by Wnt11/Fz5 signaling, secreted Frizzled-related Wnt antagonist *Tlc* and secreted frizzled related protein1 (*sfrp1*) is essential for early eye development (Cavodeassi et al., 2005). In mouse, however, inactivation of *Sfrp1*, *Sfrp2*, or both *Sfrp1* and *Sfrp2* did not cause substantial eye phenotype. In contrast, active β -catenin signaling is required for RPE differentiation, since conditional inactivation of β -catenin in mouse disturbed RPE differentiation. Interestingly, inactivation of *Wnt8b* in mouse did not cause overt alterations, probably due to compensation by other Wnts (Fotaki et al., 2010). Taken together, these data support that Wnt8b is a strong suppressor of early eye and RPC formation. In mouse, Wnt8b is repressed by Six3 at the transcriptional level to allow RPC formation (Liu et al., 2010).

3. Disease involvement

WNT8B was considered as a candidate gene for partial epilepsy condition, but the recent observation that loss of Wnt8b did not cause an overt effect on hippocampus development in mouse does not substantiate this idea. DNA sequencing of exon1 of *WNT8B* in a population of Chinese children with Hirschsprung disease (HSCR) identifies heterozygosity deletion and base replacement in WNT8B. *WNT8B* mutations that are associated with developmental abnormalities of eye formation remain to be determined.

4. Future studies

The findings that early eye formation requires antagonizing wnt8b/ β -catenin activity in zebrafish and that RPC specification requires Six3-mediated suppression of Wnt8b in mouse provide a molecular outline of the program for retinal differentiation, and are relevant to directed retinal differentiation from stem cells in the endeavor of disease modeling and cell-replacement therapy. Since Wnt8b is a negative regulator of early eye and retinal development, it is reasonable to propose that mutations in Wnt8b leading to its loss-of-function may not be the cause of developmental abnormalities in early eye formation. Rather, it is likely that any mutation(s) causing ectopic Wnt8b activity at the prospective rostro-ventral forebrain and eye primordia could interrupt early eye development. One candidate of such mutations is the *cis*-element of Wnt8b through which Six3 exerts its transcriptional repression (Liu et al., 2010), and may be relevant to genetic testing of the patients with a broad range of developmental defects in neuroretina.

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Figure 1. Structure and function of mouse Wnt-8b protein
(A) Primary structure of mouse Wnt-8b (Q9WUD6, 350 aa). Predicted cleavage site of signal peptide (arrowhead), InterPro signatures are shown. (B) Simplified two-state model for Wnt/β-catenin pathway. See text for details. (C–F) Expression of Wnt8b and Six3 in E8.5 mouse embryos. See text for details (G) Six3-mediated suppression of Wnt8b at the anterior neural plate is required for RPC specification, while active Wnt/β-catenin activity is required for RPE differentiation. ANP, anterior neural plate; EF, eye field; RPE, cells forming retinal pigment epithelium; RPC, retinal progenitor cells. Scale bar in C, 100μ.