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Mechanisms of Wnt signaling: from embryonic stem cells to dopaminergic neurons

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ABSTRACT

The ability of a cell to respond in a specific way to certain signals represents key biological phenomena governing development of multicellular organism. Cellular signaling regulates all aspects of cell biology such as proliferation, migration, differentiation, and death. Detailed understanding of mechanisms by which various signals are interpreted into certain cellular responses is crucial in order to efficiently manipulate these processes. Guiding a stem cell via specific cues to a cell type of interest, such as dopaminergic (DA) neurons, is a necessary prerequisite for cell replacement therapy (CRT) of diseases, such as Parkinson's disease (PD), where DA neurons are progressively lost. This thesis examines molecular mechanisms of action of Wnts, a group of factors providing such cues, and their functional role in midbrain development and DA neuron differentiation.

In our first study we manipulated Wnt/β-catenin signaling pathway in mouse embryonic stem cells (mESCs) to analyze its impact on mESC differentiation into DA neurons. We show that impairment of the pathway at the level of ligand (Wnt1) or receptor (LRP6) level enhances neuronal and DA differentiation of mESCs. Similarly, application of Dkk1 (Wnt/β-catenin pathway inhibitor) also increased the yield of mESC-derived DA neurons. Combined, our data demonstrate that Wnt1 and LRP6 are dispensable for mESC DA differentiation, that mESC differentiation into DA neurons is facilitated by attenuated Wnt/β-catenin signaling, and that inhibitors of Wnt/β-catenin pathway can be used to increase efficiency of DA differentiation protocols.

Earlier reports from our lab demonstrated enhancement of DA differentiation by Wnt5a, an activator of Wnt/β-catenin-independent pathways in DA cells. Thus, we focused on mechanisms of Wnt/β-catenin-independent signaling and its functional aspects in our following studies, as these were not elucidated before this thesis. We show by analyses of Wnt5a -/- mice embryos the importance of Wnt5a for proper midbrain morphogenesis. Moreover, absence of Wnt5a led to increase in proliferation of DA progenitors, accumulation of Nurr1+ precursors and attenuated differentiation of these precursors into TH+ DA neurons.

To characterize Wnt5a-mediated effect on DA differentiation we analyzed possible activation of putative downstream pathway components. We demonstrate that Wnt5a effects on DA differentiation are mediated via small GTPase Rac1, which is a downstream effector of Wnt5a/Dvl signaling in DA cells. Subsequently, we examined molecular aspects of the Wnt5a/Dvl/Rac signaling in closer detail. We demonstrate that β-arrestin is a crucial component of Wnt5a/Dvl/Rac signaling route and we show its critical role in regulation of CE movements during *Xenopus* gastrulation. Moreover, we found that specification of Wnt-mediated signaling at the level of Dvl is further controlled by phosphorylation of Dvl by casein kinases CK1 and CK2. Therefore, CK1 and CK2 act as switches between distinct branches of Wnt/β-catenin-independent signaling. Next, to get further insight into Wnt5a/Dvl-mediated activation of Rac1 we analyzed the Dvl-Rac1 interaction and performed a proteomic screen for Dvl-binding regulators of Rac1 activity. We show that Dvl and Rac1 form a complex, and the N-terminal part of Dvl mediates this interaction. Further, we demonstrate that Tiam1, a novel Dvl-binding partner found in our study, is required both for Rac1 activation in the Wnt5a/Dvl/Rac signaling branch and for DA neuron differentiation. Collectively, we identified β-arrestin, CK1, CK2, and Tiam1 as novel regulators of Wnt5a-induced signaling.

In sum, data in the presented thesis describes molecular mechanisms and functional consequences of Wnt-driven signaling pathways and pinpoints the modulation of Wnt signaling as a possible tool to improve PD therapies.

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