Wnt5a cooperates with canonical Wnts to generate midbrain dopaminergic neurons in vivo and in stem cells

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AUTHOR SUMMARY

Stem cells are considered ideal tools for cell-replacement therapy and drug-discovery assays for Parkinson’s disease, a neurodegenerative disorder affecting midbrain dopaminergic (DA) neurons. To exploit the therapeutic potential of stem cells, it is essential to elucidate the mechanisms that control the development of midbrain DA neurons and implement them in stem cells. Here, we demonstrate that two secreted developmental factors, Wnt1 and Wnt5a, cooperate to control midbrain DA neuron development in vivo. In addition, we develop two protocols in which we apply Wnts to improve the generation of midbrain DA neurons from neural and embryonic stem cells.

Wnts are a family of secreted proteins that bind to membrane receptors on cells and mediate communication either through activation of an intracellular protein, β-catenin (i.e., via the Wnt/β-catenin pathway) or through β-catenin-independent mechanisms involving other proteins such as Rac1 [i.e., via the Wnt/planar cell polarity (PCP)/Rac1 pathway] (1). Wnts regulate different aspects of midbrain DA neuron development. In cell culture, Wnts activating the Wnt/β-catenin pathway (Wnt1 and Wnt3a) primarily affect proliferation, whereas Wnts activating the Wnt/PCP/Rac1 pathway (Wnt5a) primarily induce differentiation (2). In vivo, mice lacking Wnt1 (Wnt1\textsuperscript{−/−}) mice show a dramatic loss of DA neurons (3), but those lacking Wnt5a (Wnt5a\textsuperscript{−/−}) mice unexpectedly show no DA neuron loss (4). In our study, we asked whether these two Wnts cooperate to regulate midbrain DA neuron development in vivo and whether such Wnts can be used in combination to enhance the generation of DA neurons from stem cells.

To address the first question, we generated double heterozygous (Wnt1\textsuperscript{+/-}; Wnt5a\textsuperscript{+/-}) mice and bred them to generate single-mutant (Wnt1\textsuperscript{−/−} or Wnt5a\textsuperscript{−/−}) and double-mutant (Wnt1\textsuperscript{−/−}; Wnt5a\textsuperscript{−/−}) mice.

First we analyzed the development of midbrain DA neurons in Wnt1\textsuperscript{−/−} mice. Surprisingly, midbrain DA neurons were absent in the most ventral domain of the midbrain (i.e., the floorplate), but a few cells were found ectopically in a lateral position (i.e., in the basal plate). Several factors used to identify cells in the DA lineage (Lmx1a, Ngn2, and Nurr1) also were abnormally expressed in cells occupying lateral positions. Accordingly, the floorplate of Wnt1\textsuperscript{−/−} mice showed reduced numbers of progenitor cells, and no new neurons were generated, indicating that neurogenesis—the process of generating new neurons—was blocked.

Next, we examined Wnt1\textsuperscript{−/−}; Wnt5a\textsuperscript{−/−} mice and found they share with Wnt5a\textsuperscript{−/−} mice a shortening of the anterior–posterior axis and with Wnt1\textsuperscript{−/−} mice a partial midbrain-hindbrain deletion. However, upon closer scrutiny, we discovered that Wnt1 and Wnt5a interact in a complex manner to promote the generation of DA neurons in vivo. Indeed, deletion of both Wnts partially compensated the proliferation defect seen in Wnt1\textsuperscript{−/−} mice, indicating that Wnt1 and Wnt5a compete in proliferation. However, both Wnts cooperated in morphogenesis and in the generation of postmitotic Nurr1\textsuperscript{+} dopamine precursors and tyrosine hydroxylase-positive DA neurons, because deletion of both Wnts worsened both the decrease in postmitotic cells seen in Wnt1\textsuperscript{−/−} mice and the morphogenesis defect observed in Wnt5a\textsuperscript{−/−} mice (4).

Based on our results, which demonstrate cooperation between Wnt1 and Wnt5a to promote midbrain DA neuron development, we developed a three-step differentiation protocol and applied purified Wnt proteins to midbrain neural stem cells and embryonic stem cells in a specific temporal sequence (Fig. P1). First, cells were grown in the presence of a Wnt capable of activating Wnt/β-catenin (Wnt3a, because currently available Wnt1 is


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inactive) to stimulate the cells to proliferate. Next cells were grown in the absence of Wnt for 2 or 3 d. Finally, cells were differentiated in the presence of Wnt5a. This protocol increased the number of DA neurons derived from ventral midbrain neural stem cells and the number of midbrain DA neurons derived from mouse embryonic stem cells. These results show that, by understanding the mechanisms that control midbrain DA neuron development, it is possible to improve current protocols for the DA differentiation of stem cells.

Our results show that coordinated Wnt actions promote DA neuron development in vivo and, particularly, that Wnt1 and Wnt5a cooperate to enhance DA neurogenesis and differentiation. Moreover, we describe a protocol for the improved DA differentiation of stem cells in which the coordinated application of Wnt proteins to neural or embryonic stem cells significantly increases the yield of midbrain DA neurons. Our Wnt-based protocols therefore could contribute to the development of assays for drug discovery and cell-replacement therapy for Parkinson’s disease.