BMP2 AND GDF5 INDUCE NEURONAL DIFFERENTIATION THROUGH A SMAD DEPENDANT PATHWAY IN A MODEL OF HUMAN MIDBRAIN DOPAMINERGIC NEURONS.

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Introduction

Ventral midbrain (VM) dopaminergic (DA) neurons project to the dorsal striatum via the nigrostriatal pathway to regulate voluntary movements, and their progressive loss causes the motor dysfunction of Parkinson’s disease (PD). Despite recent progress in the understanding of VM DA neurogenesis, the factors regulating nigrostriatal pathway development remain largely unknown (Figure 1).

The bone morphogenetic protein (BMP) family regulate neurite growth in the developing nervous system (NS), and two related members of this family, BMP2 and growth differentiation factor 5 (GDF5), promote neurite growth in cultured VM DA neurons. However, the molecular (signaling pathway(s)) and cellular (direct neuronal or indirect via glial cells) mechanisms of their effects on DA neurons remain to be elucidated.

During development, GDF5 and BMP2 activate a canonical signalling pathway (Figure 2) involving type I and type II BMP receptors (BMPRs). When bound to by BMPs, the BMPRII activates the BMPRI that then phosphorylates Smad 1/5/8. These R-Smads translocate to the nucleus (mediated by Smad4) to modulate gene expression.

Aim of study

Using the SH-SH5Y cell line as a model of human midbrain DA neurons, we investigate the neurite outgrowth-promoting ability of GDF5 and BMP2, and determine whether the canonical BMP-Smad signalling pathway mediates their neurotrophic effects.

Methods

Results

BMP2 and GDF5 induce neuronal differentiation of SH-SH5Y cells.

SH-SH5Y cells express the mRNA and protein of the BMPRIb and BMPRII receptors, the Smad1, Smad5 and Smad8 R-Smads, and Smad4 of the Smad signalling pathway, as shown by RT-PCR and immunocytochemistry (Fig. 3a-c).

BMP2 and GDF5 significantly reduce the proliferation, as shown by MTT Assay (Fig. 3d), and significantly increase the total neurite length (Fig. 3d-e) of SH-SH5Y cells.

BMPRI activation is required for BMP2- and GDF5-induced neuronal differentiation and Smad signalling in SH-SH5Y cells.

Dorsomorphin, an inhibitor of BMPRI, pre-treatment prevents BMP2- and GDF5-induced reduction in SH-SH5Y cell proliferation (Fig. 5a).

Dorsomorphin pre-treatment prevents BMP2- and GDF5-induced neurite growth (Fig. 5b-c).

Pre-treatment with dorsomorphin inhibits the activation of Smad 1/5/8 signalling by BMP2 and GDF5 in SH-SH5Y cells (Fig. 5d-e).

BMP2 and GDF5 activate the canonical Smad 1/5/8 signalling pathway in SH-SH5Y cells.

BMP2 significantly increases nuclear levels of phospho-Smad 1/5/8 at 5, 15, 30, 60 and 120 min (Fig. 4a-b), as determined by densitometric analysis.

GDF5 significantly increases nuclear levels of phospho-Smad 1/5/8 at 60 and 120 min (Fig. 4c-d), as determined by densitometric analysis.

siSmad4 knocks-down SH-SH5Y Smad4 expression (Fig. 7a-c).

Smad4 overexpression or knockdown does not affect the SH-SH5Y neurite growth, in the absence of active BMP-Smad signalling (Fig. 7d).

Smad4 knockdown by siSmad4 prevents the caBMPRIb-induced neurite growth of SH-SY5Y (Fig. 7e-f).

Smad4 knockdown by siSmad4 prevents BMP2- and GDF5-induced neurite growth of SH-SY5Y cells (Fig. 8a-b).

Smad transcriptional activity is required for BMP2- and GDF5-induced, BMPRIb-mediated neurite growth of SH-SH5Y cells.

The active BMPRIb activates the Smad signalling pathway and mimics the neurite growth-promoting effects of BMP2 and GDF5 in SH-SH5Y cells.

Transfection of SH-SH5Y cells with a constitutively active BMPRIb results in a significant increase in neurite length of SH-SH5Y cells (Fig. 6a-b).

SH-SH5Y cells expressing a constitutively active (ca) BMPRIb have significantly increased nuclear phospho-Smad 1/5/8 levels (Fig. 6c-d).

siSmad4 knocks-down SH-SH5Y Smad4 expression (Fig. 7a-c).

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Smad4 knockdown by siSmad4 prevents BMP2- and GDF5-induced neurite growth of SH-SY5Y cells (Fig. 8a-b).

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Conclusion

The downstream molecular mechanisms mediating the direct neurite growth-promoting effects of GDF5 and BMP2 are dependent upon BMPRIb-mediated activation of the canonical Smad 1/5/8 signalling pathway.