

Title	Roles of FGF20 in dopaminergic neurons and Parkinson's disease.
Author(s)	Itoh, Nobuyuki; Ohta, Hiroya
Citation	Frontiers in molecular neuroscience (2013), 6
Issue Date	2013-05
URL	http://hdl.handle.net/2433/175246
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Type	Journal Article
Textversion	publisher



Roles of FGF20 in dopaminergic neurons and Parkinson's disease

Nobuyuki Itoh* and Hiroya Ohta

Department of Genetic Biochemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Kyoto, Japan

Edited by:

Volker Eulenburg, University of Erlangen1054, Germany

Reviewed by:

Marcos R. Costa, Federal University of Rio Grande do Norte, Brazil

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*Correspondence:

Nobuyuki Itoh, Department of Genetic Biochemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Yoshida-shimoadachi, Sakyo, Kyoto 606-8501, Japan
e-mail: itohnobu@pharm.kyoto-u.ac.jp

The fibroblast growth factor (FGF) family comprises 22 members with diverse functions in development and metabolism. *Fgf20* was originally identified as a new *Fgf* preferentially expressed in the substantia nigra pars compacta (SNpc). *Fgf20*, which acts on proximal cells, significantly enhanced the survival of cultured dopaminergic neurons by activating the mitogen-activated protein kinase (MAPK) pathway through *Fgf* receptor 1c. In the rat model of Parkinson's disease, *Fgf20* afforded significant protection against the loss of dopaminergic neurons. The significant correlation of Parkinson's disease with single-nucleotide polymorphisms in *FGF20* indicates that the genetic variability of *FGF20* can be a Parkinson's disease risk. Neural and embryonic stem (ES) cells have been considered as cell resources for restorative transplantation strategies in Parkinson's disease. *Fgf20* promoted the differentiation of these stem cells into dopaminergic neurons, which attenuated neurological symptoms in animal models of Parkinson's disease. These findings indicate the importance of FGF20 for the differentiation and survival of dopaminergic neurons and the etiology and therapy of Parkinson's disease.

Keywords: dopaminergic neurons, Fgf, Fgf20, Parkinson's disease, stem cells, SNP

INTRODUCTION

Fibroblast growth factors (FGFs) are polypeptides with diverse functions in development, metabolism, and neural activities. The FGF family comprises 22 members, which have been classified as paracrine, endocrine, and intracrine FGFs by their mechanisms of action. Most FGFs are paracrine FGFs that act as local signaling molecules (Itoh and Ornitz, 2011). *Fgf20* was originally identified as a new *Fgf* preferentially expressed in the substantia nigra pars compacta (SNpc). *Fgf20*, a paracrine *Fgf*, with neurotrophic activity in cultured dopaminergic neurons (Ohmachi et al., 2000, 2003) has been suggested to play important roles in the development of dopaminergic neurons (Grothe et al., 2004; Takagi et al., 2005; Correia et al., 2007; Shimada et al., 2009). In addition, *FGF20* mutations may result in Parkinson's disease (van der Walt et al., 2004; Satake et al., 2007; IPDGC, 2011; Pan et al., 2012; Pihlström et al., 2013; Wang et al., 2013). As these findings indicate that FGF20 may provide useful clues on the etiology and therapy of Parkinson's disease, a succinct review on the roles of FGF20 in dopaminergic neurons and Parkinson's disease has been provided. In this review, we refer to the human and rodent orthologs as *FGF20* and *Fgf20* according to the Human Genome Organization and the Mouse Genome Informatics, respectively.

IDENTIFICATION OF FGF20

Fgf20, originally identified in the rat brain, encodes a secreted protein of 212 amino acids (Ohmachi et al., 2000). The *FGF* gene family comprising 22 members has been classified into 7 subfamilies; *FGF1/2*, *FGF4/5/6*, *FGF3/7/10/22*, *FGF8/17/18*, *FGF9/16/20*, *FGF11/12/13/14*, and *FGF19/21/23*. FGF20 is a member of the *FGF9/16/20* subfamily, which is a paracrine *Fgf* (Figure 1) (Itoh and Ornitz, 2011).

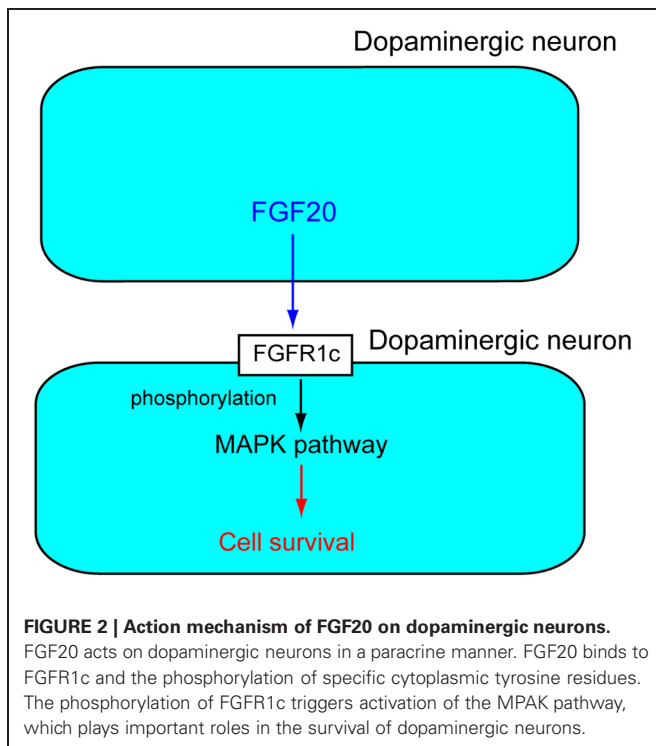
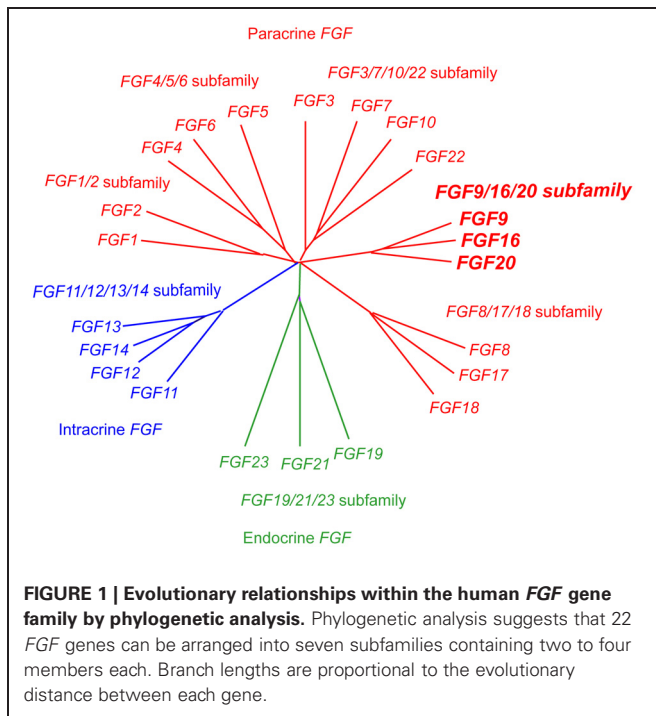
FGF20 IN DOPAMINERGIC NEURON SURVIVAL

As paracrine FGFs are local signal molecules, *Fgf20* is expected to act on dopaminergic neurons in the SNpc in a paracrine manner. However, as both *Fgf20* and *Fgfr1* are expressed in most dopaminergic neurons in the SNpc, *Fgf20* may act on them in an autocrine/paracrine manner. *Fgf20* was shown to significantly enhance the survival of cultured rat dopaminergic neurons (Ohmachi et al., 2000, 2003).

Paracrine FGF signaling is mediated by the activation of FGFR. Paracrine FGFs bind to FGFRs and induce the phosphorylation of specific cytoplasmic tyrosine residues, which triggers the activation of cytoplasmic signal transduction pathways. Major FGF/FGFR-dependent signaling was shown to be mediated by the mitogen-activated protein kinase (MAPK) and phospholipase-C γ pathways (Thisse and Thisse, 2005).

FGFRs are receptor tyrosine kinases with an extracellular ligand-binding domain, which comprises three immunoglobulin-like domains (I, II, and III). There are seven major FGFR proteins including FGFRs1b, 1c, 2b, 2c, 3b, 3c, and 4, which are generated from four functional *FGFR* genes, *FGFR1-FGFR4* by alternative splicing (Zhang et al., 2006). *Fgfr1c* is abundantly expressed in dopaminergic neurons in the SNpc. *Fgf20* binds to *Fgfr1c* with high affinity. Experiments with the *Fgfr* inhibitor SU5402 or MAPK pathway inhibitor PD98059 indicate that activation of the MAPK pathway by *Fgf20* through *Fgfr1c* is essential for the survival of dopaminergic neurons in the SNpc (Figure 2) (Ohmachi et al., 2003).

Calbindin-negative dopaminergic neurons are preferentially lost in Parkinson's disease. *Fgf20* almost completely rescued rat calbindin-negative midbrain dopaminergic neurons from the toxicity of 6-hydroxydopamine and stress-induced cytosolic



dopamine, and promoted dopamine release in calbindin-negative dopaminergic neurons by activating Fgfr1 followed by its downstream cascade activation. These results show that Fgf20 protects the specific midbrain neuron type at most risk in Parkinson's patients (Murase and McKay, 2006).

In the unilateral, 6-hydroxydopamine lesion rat model of Parkinson's disease, supranigral infusion of Fgf20 afforded significant protection against the loss of dopaminergic neurons in the SNpc and striatum. Protection of the nigrostriatal tract was accompanied by the significant preservation of gross locomotion and fine motor movements and the reversal of apomorphine-induced contraversive rotations. These results imply the potential neuroprotective role of Fgf20 in this disease (Sleeman et al., 2012).

FGF20 IN PARKINSON'S DISEASE

Parkinson's disease is a common neurodegenerative disorder. The inability to control movement in patients with this disease has been attributed to the severe loss of dopaminergic neurons within the substantia nigra. Environmental and genetic sources act together in the disease cascade.

FGF20 has been mapped to 8p21.3–8p22, which is within an area of Parkinson's disease linkage. To test whether *FGF20* genetic variability was a risk factor for Parkinson's disease, five single-nucleotide polymorphisms (SNPs) lying in *FGF20* were examined in a large family study. The highly significant correlation of Parkinson's disease with one SNP located in the intron and two SNPs in the 3' regulatory region was revealed, which indicated that *Fgf20* genetic variability is a risk factor for Parkinson's disease (van der Walt et al., 2004). In addition, *FGF20* genetic variability was shown to be a risk factor for Parkinson's disease in Japanese and Chinese populations (Satake et al., 2007; Pan et al., 2012), while, was not a risk factor for Parkinson's disease in Finnish and Greek populations (Clarimon et al., 2005). The discrepancy between these results remains to be elucidated.

The SNP in the 3' non-coding region of *FGF20* can be a risk factor for Parkinson's disease. The risk allele disrupts a binding site for microRNA-433, increasing *FGF20* mRNA translation. This increase in *FGF20* mRNA translation has been correlated with increased α -synuclein expression. As α -synuclein is the principal component of filamentous Lewy bodies, the defining pathological hallmark of Parkinson's disease, these findings suggest a novel mechanism of action for the risk of Parkinson's disease (Wang et al., 2008). In addition, *FGF20* and α -synuclein were also shown to be associated with sporadic Parkinson's disease (Mizuta et al., 2008). However, no association between the SNP in *FGF20*, microRNA-433, or α -synuclein expression and Parkinson's disease have been reported (Wider et al., 2009; de Mena et al., 2010). The discrepancy between these results remains to be elucidated.

The genetic variability of the monoamine oxidase B gene (*MAOB*) has also been suggested as a risk factor for Parkinson's disease. Both *FGF20* and *MAOB* are in the dopamine bio-pathway. SNP variants in *FGF20* and *MAOB* show evidence of statistical interactions, which emphasizes the importance of considering them jointly in the genetic analysis of Parkinson's disease, and illustrates the potential patterns of biological interactions contributing to the risk of Parkinson's disease (Gao et al., 2008).

A genome-wide association study (GWAS) to examine many common genetic variants was conducted in different individuals to identify any variant associated with a trait. The GWAS typically focused on associations between SNPs and traits such as major

diseases. *FGF20* was shown to be a risk factor for Parkinson's disease by the GWAS (IPDGC, 2011; Pihlström et al., 2013; Wang et al., 2013).

FGF20 IN THE NEURAL DIFFERENTIATION OF STEM CELLS INTO DOPAMINERGIC NEURONS

Neural stem (NS) cells are multipotent cells characterized by their capability to differentiate into neurons, astrocytes, and oligodendrocytes, and have been considered as cell resources for restorative transplantation strategies in Parkinson's disease. *Nurr1* is a transcription factor of the thyroid hormone/retinoic acid nuclear receptor superfamily that is required for the induction of dopaminergic neurons. However, *Nurr1* alone is not sufficient to induce a dopaminergic phenotype in NS cells. A co-culture of *Nurr1*-transfected NS cells with Schwann cells over-expressing *Fgf20* was shown to induce dopaminergic neurons in NS cells. Differentiated *Nurr1*-NS cells retained both neuronal morphology and tyrosine hydroxylase expression after transplantation into the striatum of 6-hydroxydopamine-lesioned rats. However, neuritogenesis was only observed after postnatal grafts. These results suggest that *Fgf20* promotes the differentiation of *Nurr1*-NS cells into dopaminergic neurons and that additional factors are required for the efficient differentiation of dopaminergic neurons in the adult brain (Grothe et al., 2004).

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the preimplantation blastocyst. These cells have many of the characteristics required of a cell source for cell-replacement therapy, including proliferation and differentiation capacities. ES cells are also promising donor cell sources for cell-replacement therapy in Parkinson's disease. FGF20 acts synergistically with FGF2 to increase the number of dopaminergic neurons in primate ES cell-derived neurospheres composed of neural progenitors. Dopaminergic neurons generated from primate ES cells were transplanted into 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated (MPTP-treated) primates, a primate model for Parkinson's disease. Behavioral studies and functional imaging revealed that the transplanted dopaminergic cells functioned as dopaminergic neurons and attenuated MPTP-induced neurological symptoms (Takagi et al., 2005).

Parthenogenesis has attracted attention as an alternative method to derive ES cells that does not involve the destruction of viable embryos. Transplantation of dopaminergic neurons generated from parthenogenetic primate ES cells restored motor function in hemi-Parkinsonian, 6-hydroxy-dopamine-lesioned rats. Exposure to FGF20, along with WNT5a and FGF2, at the final

stage of *in vitro* differentiation enhanced the maturation and *in vivo* survival of dopaminergic neurons and, correspondingly, the extent of motor recovery in transplanted animals (Sanchez-Pernaute et al., 2008). Induced pluripotent stem (iPS) cell-derived dopaminergic neurons were also shown to integrate into the striatum of Parkinsonian rats with behavioral improvements (Gibson et al., 2012). However, experiments on iPS cells using FGF20 have not been reported.

Neuronal differentiation in human ES cells was induced by co-culturing with PA6 mouse stromal cells. The number of tyrosine hydroxylase-expressing neurons significantly increased in culture medium supplemented with FGF20. Cultured cells also expressed other midbrain dopaminergic markers, which suggests that some differentiate into midbrain dopaminergic neurons. However, FGF20 has no effect on the size of the soma area or neurite length of dopaminergic neurons. FGF20 significantly reduced the proportion of cells undergoing cell death. These results indicate that FGF20 specifically increased the yield of dopaminergic neurons from human ES cells grown on PA6 feeder cells, and at least part of this effect was due to a reduction in cell death (Correia et al., 2007). In addition, FGF20 along with FGF2 enhanced dopaminergic neuron differentiation from human ES cell-derived neural progenitor cells directly without co-culturing with PA6 cells (Shimada et al., 2009).

CONCLUSIONS

Fgf20 is expressed in the SNpc of the midbrain. *Fgf20* significantly enhances the survival of cultured dopaminergic neurons in a paracrine manner. In the rat model of Parkinson's disease, *Fgf20* affords significant protection against the loss of dopaminergic neurons. The significant correlation of Parkinson's disease with SNPs within *FGF20* indicated that *FGF20* genetic variability is a risk factor for Parkinson's disease. *Fgf20* promotes differentiation of cultured cells into dopaminergic neurons, and attenuated neurological symptoms in animal models of Parkinson's disease. These findings indicate the importance of FGF20 in both the differentiation and survival of dopaminergic neurons and the etiology and therapy of Parkinson's disease. Further studies on FGF20 will provide useful clues on the etiology and therapy of Parkinson's disease.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 April 2013; paper pending published: 27 April 2013; accepted: 16 May 2013; published online: 31 May 2013.

Citation: Itoh N and Ohta H (2013) Roles of FGF20 in dopaminergic neurons and Parkinson's disease. *Front. Mol. Neurosci.* 6:15. doi: 10.3389/fnmol.2013.00015

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