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Review

Midbrain dopaminergic neurons: A review of the molecular circuitry that regulates their development

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ABSTRACT

Dopaminergic (DA) neurons of the ventral midbrain (VM) play vital roles in the regulation of voluntary movement, emotion and reward. They are divided into the A8, A9 and A10 subgroups. The development of the A9 group of DA neurons is an area of intense investigation to aid the generation of these neurons from stem cell sources for cell transplantation approaches to Parkinson's disease (PD). This review discusses the molecular processes that are involved in the identity, specification, maturation, target innervation and survival of VM DA neurons during development. The complex molecular interactions of a number of genetic pathways are outlined, as well as recent advances in the mechanisms that regulate subset identity within the VM DA neuronal pool. A thorough understanding of the cellular and molecular mechanisms involved in the development of VM DA neurons will greatly facilitate the use of cell replacement therapy for the treatment of PD.

Introduction

In the adult CNS, almost 75% of all dopaminergic neurons reside in the ventral midbrain (VM), with 400,000–600,000 found in the human VM and 20,000–30,000 in the mouse VM (Blum, 1998; German et al., 1983; Pakkenberg et al., 1991). During embryonic development, these DA neurons are generated in the floor plate region of the mesencephalon (Ono et al., 2007), and give rise to three distinct clusters of VM DA neurons which ultimately develop into anatomically and functionally distinct entities termed the A8, A9 and A10 groups. The A9 cluster gives rise to the substantia nigra pars compacta (SNc), whose neurons project to the dorsal striatum via the nigrostriatal pathway. These neurons and their striatal projections are required for the control of voluntary movement, and the loss of these neurons is the pathological hallmark of Parkinson's disease (PD), which is a neurodegenerative disorder characterised by impaired motor function (Lees et al., 2009; Toulouse and Sullivan, 2008). The other groups of DA neurons, the A10 and A8 clusters, develop into the ventral tegmental area (VTA) and the retrorubal field (RRF), respectively, whose neurons innervate the ventral striatum and the prefrontal cortex via the mesocorticolimbic system, and are involved in the regulation of emotion and reward (Tzschentke and Schmidt, 2000). Altered/defective neurotransmission of the mesocorticolimbic DA system has been associated with the development of schizophrenia, drug addiction and depression (Meyer-Lindenberg et al., 2002; Robinson and Berridge, 1993).

Interestingly, the A9 group of SNc DA neurons, which undergo progressive degeneration in PD, are particularly vulnerable to cell death in comparison to the other VM DA neuronal populations (Alavian et al., 2008; Betarbet et al., 2000; Farrer, 2006; McNaught et al., 2004). The anatomical, functional and apparent sensitivity differences between these three populations of VM DA neurons likely results from subtle developmental differences during their ontogeny. However, little is known regarding the molecular mechanisms that regulate the phenotypic and functional diversities between these VM DA neuronal populations. Given the involvement of A9 DA neurons in PD, an intensive research effort over the last five decades has focused on identifying the molecules and mechanisms that regulate their development. This information is vital to advance efforts to generate SNc DA neurons from stem cells for application in cell replacement therapy for PD. Through the mutation of specific genes, and the subsequent analysis of VM DA neurogenesis and development, a number of molecular pathways have been shown to play key roles in the

Abbreviations: A/P, anterior–posterior; BDNF, brain-derived neurotrophic factor; DA, dopaminergic/dopamine; DAT, dopamine transporter; D/V, dorso-ventral; E, embryonic day; *En1/2*, *Engrailed-1/2*; FGF8, fibroblast growth factor 8; Fzd, frizzled; GDNF, glial cell line-derived neurotrophic factor; MFB, medial forebrain bundle; NPs, neural progenitor(s)/precursor(s); NSCs, neuroepithelial/neural stem cells; P, postnatal day; PD, Parkinson's disease; RRF, retrorubal field; Shh, sonic hedgehog; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase; VM, ventral midbrain/mesencephalon; VTA, ventral tegmental area; VZ, ventricular zone.

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development of VM DA neurons. This review discusses the 'normal' developmental programme that regulates VM DA neurogenesis, including the cellular and molecular determinants involved in their regional specification, induction, differentiation and maturation.

Early patterning of the ventral mesencephalon

The first key steps in VM DA generation are the early patterning events which lead to the formation of the VM region. During gastrulation, the dorsal ectoderm is restricted towards a neural fate in response to signals arising from the Speamann organiser (Harland, 2000; Hemmati-Brivanlou and Melton, 1997; Liu and Niswander, 2005). The resulting neural plate is then subdivided into restricted domains and subsequently closes to form the neural tube, which is specified by graded signals along the anterior–posterior (A/P) and dorso-ventral (D/V) axes (Puelles, 2001; Simon et al., 1995; Ulloa and Briscoe, 2007). The development of the VM region relies on appropriate A/P and D/V patterns of gene expression which are regulated by signals arising from two key structures in the early embryo: the floor plate of the midbrain and the isthmus organiser. Organisation of the VM region is initiated upon formation of these signalling centres.

The floor plate is present along the length of the neural tube and secretes the sonic hedgehog (Shh) signalling protein from around embryonic day (E) 8.5 onwards in the mouse (Echelard et al., 1993; Ho and Scott, 2002; Hynes et al., 1995a). Interestingly, the spatiotemporal expression pattern of *Shh* in the VM has been shown to contribute to the diverse populations of VM DA neurons, with the 'early medial pool' giving rise primarily to VTA, and very few SNc, DA neurons and the 'later intermediate pool' giving rise to DA neurons of all three subgroups, but largely contributing to the SNc (Joksimovic et al., 2009a). In the floor plate, the bHLH (basic helix–loop–helix) transcription factor Hes1 (also expressed by the isthmus organiser) has been shown to suppress proneural gene expression and induce cell cycle exit (Baek et al., 2006; Ono et al., 2010). Null mutation of *Hes1* results in a transient increase in the number of VM DA neurons between E11.5 and E12.5, followed by a significant reduction in their number from E13.5, compared to the wild type (Kameda et al., 2011). Interestingly, another bHLH transcription factor expressed in the floor plate, Nato3, has been shown to repress *Hes1* expression, and mutation of *Nato3* has been shown to result in a reduction in the number of VM DA neurons generated due to unchecked Hes-1-mediated suppression of proneural genes and the induction of cell cycle arrest (Ono et al., 2010).

The isthmus organiser is a unique signalling centre that separates the midbrain from the hindbrain and is necessary for the development of both of these brain regions (Liu and Joyner, 2001; Rhinn and Brand, 2001). The correct positioning of the isthmus organiser at the midbrain-hindbrain boundary is dependent on the mutual repression of two opposing homeodomain transcription factors: *Otx2* and *Gbx2* (Martinez-Barbera et al., 2001). *Otx2* is expressed in the forebrain and midbrain of the developing anterior neural tube (Acampora et al., 1997; Matsuo et al., 1995; Simeone et al., 1992), while *Gbx2* is expressed more posteriorly in the anterior hindbrain (Wassarman et al., 1997). *Gbx2* expression at the posterior border limits *Otx2* expression which creates the sharp boundary between the midbrain and the hindbrain (Millet et al., 1999).

Fibroblast growth factor 8 (FGF8) is a diffusible factor secreted by the isthmus organiser (Rhinn and Brand, 2001), from around E8 until at least E12.5 in the mouse midbrain-hindbrain boundary (Crossley and Martin, 1995). Surprisingly, although *Otx2* and *Gbx2* are critical for the correct positing of the isthmus organiser, they are not required for the expression of *FGF8*, or for the induction of other isthmus organiser-genes, however they are essential for the correct positioning of the expression domains of these genes (Brodski et al., 2003; Liu and Joyner, 2001). This is highlighted by studies showing that if the position of the isthmus organiser is moved caudally as a result of ectopic *Otx2* expression in hindbrain, there is an increase in the number of VM DA neurons (Brodski et al., 2003). Similarly if its position is moved rostrally by depleting *Otx2* in the midbrain, there is a decrease in the number of VM DA neurons (Brodski et al., 2003), demonstrating the critical importance of isthmus organiser positioning for normal VM DA generation.

As *Otx2*- and *Gbx2*-dependent sharpening of the borders of the isthmus is occuring, a second group of transcription factors begin to be expressed in the isthmus organiser. These include the paired box gene *Pax2* (Urbanek et al., 1997), the lim-homeodomain factor *Lmx1b* (Adams et al., 2000; Smidt et al., 2000), the secreted glycoprotein *Wnt1* (Adams et al., 2000; Crossley and Martin, 1995, Davis and Joyner, 1988; Wilkinson et al., 1987), and *Engrailed-1* (*En1*) (Davis and Joyner, 1988). Of these, *Pax2* is required for the induction of *FGF8* expression by the isthmus, whereas *Wnt1* and *En1* function cooperatively with *Otx2* and *Gbx2* to further refine the position of the expression domain of *FGF8* at the isthmus (Ye et al., 2001).

Shortly after the induction and positioning of *FGF8* expression, *Engrailed-2* (*En2*) and *Pax5* start to be expressed in the midbrain-hindbrain boundary. These genes play critical roles in the regional specification of the VM, and homozygous mutant mice null for *Otx2* (Acampora et al., 1995; Ang et al., 1996), *Wnt1* (McMahon and Bradley, 1990; Prakash et al., 2006), *Pax2* and *Pax5* (double mutant) (Schwarz et al., 1997), *En1* and *En2* (double mutant) (Liu and Joyner, 2001; Simon et al., 2001), or *Lmx1b* (Smidt et al., 2000) all display major VM defects, including partial or total loss of VM DA neurons (see Table 1).

Identity of ventral midbrain dopaminergic neural precursors

Once the appropriate patterning of the VM region has occurred, a developmental programme involving a sequential pattern of gene expression establishes the identity of VM DA neural precursors (NPs) that ultimately generate VM DA neurons (Fig. 1). The identity of these VM DA NPs has been the focus of intensive research in recent years, largely due to their potential to be used as a cell source to generate DA neurons for cell replacement therapy in PD (Kim, 2011; Morizane et al., 2008; Toulouse and Sullivan, 2008).

The origin of VM DA NPs has been debated for many years, with regions such as the diencephalon (Marin et al., 2005), isthmus (Marchand and Poirier, 1983) and VM basal plate (Hynes et al., 1995a, 1995b) emerging as potential candidates. Despite this research, the precise identity of VM DA NPs remained elusive until recently, when a study showed that floor plate cells in the murine VM become neurogenic and subsequently give rise to DA neurons (Ono et al., 2007). This discovery was surprising as the floor plate was thought to consist of specialised non-neurogenic glial type cells that were largely involved in ventralizing the neural tube, mainly by secreting Shh (Fuccillo et al., 2006; Jessell, 2000; Placzek and Briscoe, 2005). This role in ventralisation seems to remains the main function for floor plate cells caudal to the midbrain, as the hindbrain floor plate has been shown to be non-neurogenic (Joksimovic et al., 2009b; Ono et al., 2007). However, the VM floor plate is different to its caudal counterparts and attains neurogenic potential. Ono et al. (2007) demonstrated that *Otx2*, which is critical for the positioning of the isthmus organiser, is also essential for the neurogenic potential of VM floor