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Review

Dissecting the role of Engrailed in adult dopaminergic neurons – Insights into Parkinson disease pathogenesis

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ABSTRACT

The homeoprotein Engrailed (Engrailed-1/Engrailed-2, collectively En1/2) is not only a survival factor for mesencephalic dopaminergic (mDA) neurons during development, but continues to exert neuroprotective and physiological functions in adult mDA neurons. Loss of one En1 allele in the mouse leads to progressive demise of mDA neurons in the ventral midbrain starting from 6 weeks of age. These mice also develop Parkinson disease-like motor and non-motor symptoms. The characterization of En1 heterozygous mice have revealed striking parallels to central mechanisms of Parkinson disease pathogenesis, mainly related to mitochondrial dysfunction and retrograde degeneration. Thanks to the ability of homeoproteins to transduce cells, En1/2 proteins have also been used to protect mDA neurons in various experimental models of Parkinson disease. This neuroprotection is partly linked to the ability of En1/2 to regulate the translation of certain nuclear-encoded mitochondrial mRNAs for complex I subunits. Other transcription factors that govern mDA neuron development (e.g. Foxa1/2, Lmx1a/b, Nurr1, Otx2, Pitx3) also continue to function for the survival and maintenance of mDA neurons in the adult and act through partially overlapping but also diverse mechanisms.

1. Introduction

Mesencephalic dopaminergic (mDA) neurons in the ventral midbrain are the main source of dopamine (DA) in the adult brain. Dysfunctions in the signaling of this neurotransmitter is involved in several neurological and psychiatric disorders [1–3]. In recent years, tremendous progress has been made in dissecting the genetic networks and signaling pathways that govern various steps of mDA neuron development such as patterning, induction and specification of mDA progenitors, and their subsequent differentiation into mature mDA neurons. These studies mainly performed using transgenic mouse models have revealed that mDA neuron ontogenesis is controlled by the concerted action of several transcription factors including Engrailed-1/Engrailed-2 (collectively Engrailed or En1/2), Foxa1/2, Lmx1a/b, Nurr1, Otx2 and Pitx3, and growth factors or morphogens such as Shh, Fgf8, Tgf-ß and Wnt1 [4–6]. It was shown for instance that *En1/2* alleles are critically required for the survival and maintenance of mature mDA neurons during late embryonic life in a dose-dependent manner [7–10]. Other transcription factors such as Nurr1 and Pitx3 play key roles in the acquisition of a mature mDA neuron identity during development by regulating the expression of several mDA neuron-specific markers such as tyrosine hydroxylase (TH) and dopamine transporter (DAT) [5,6].

Interestingly, a number of recent studies have shown that several transcription factors, which control mDA neuron development, display continued expression in adult mDA neurons and are required for the maintenance of these neurons throughout life [11,12]. These findings are particularly interesting in the context of Parkinson disease (PD), since possible genetic links between some of these transcription factors (e.g. EN1/2, NURR1, PITX3, LMX1A/B) and PD have been reported [13–17], although these findings await further confirmations. Deciphering the role played by these transcription factors in mDA neurons during development

Abbreviations: En1/2, Engrailed-1/Engrailed-2; DA, dopamine; DAT, dopamine transporter; mDA, mesencephalic dopaminergic; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; PD, Parkinson disease; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; VTA, ventral tegmental area

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and in the adult, and elucidating the underlying mechanisms are an emerging field in PD research.

This review is mainly focused on the homeoprotein Engrailed, which belongs to the class of DNA binding homeodomain containing transcription factors. The expression of *En1/2* during development starts early at embryonic day E8 in a patch of cells in the anterior neuroepithelium in the midbrain hindbrain boundary region. The extension of brain expression of *En1/2* changes during the course of development and then confines to midbrain and hindbrain structures in the adulthood [18]. In the ventral midbrain, *En1/2* expression in the adult becomes highly restricted to mDA neurons of the substantia nigra pars compacta (SNpc), which preferentially degenerate in PD, and of the ventral tegmental area (VTA) [7,8]. Interestingly, recent work, using genetic and toxicological mouse models, support the idea that EN1/2 might be in the PD pathway [19–22]. We review here the lessons emerging from these studies, which bring important insights into mDA neuron physiology, our understanding of PD pathogenesis and which open new perspectives for PD therapeutics.

2. Engrailed and PD connection

2.1. Hallmarks of PD

The main hallmark of PD, which represents the second most common neurodegenerative disorder after Alzheimer's disease, is the slow and progressive loss of mDA neurons of the SNpc [1–3]. These neurons project to the dorsal striatum and form the nigrostriatal pathway, involved in the control of voluntary movements. The massive loss of these neurons ultimately results in severe striatal DA deficiency, which leads to classical PD-associated motor symptoms such as tremor, rigidity, bradykinesia and postural instability. Another hallmark of PD is the presence of intraneuronal inclusions mainly containing α-synuclein protein aggregates within the cell body (Lewy bodies) or processes (Lewy neurites). Age is the major risk factor for the development of PD [23], and the prevalence and incidence greatly increase after the age of 80 years. Exposure to environmental factors (i.e. pesticides) can also increase the risk to develop the disease [3]. Diagnosis of PD occurs with the onset of motor symptoms, but this can be preceded by a pre-motor or prodromal phase of 20 years or more [3]. It has been assumed for long time that the first motor symptoms appear when more than 80% of striatal DA is lost [1]. However, a reassessment of all available data suggests that the loss of SNpc mDA neurons remains asymptomatic until 30% of mDA neuron cell bodies and 50–60% of axonal terminals are lost [24]. In any case, adaptive changes in DA terminals and postsynaptic striatal neurons appear to compensate for significant losses of striatal DA to preserve motor behavior. It was recently shown that toxigenetic ablation of mDA neurons in the mouse following targeted expression of the diphtheria toxin A gene during development results in a depletion of more than 95% of striatal DA and this does not lead to any motor symptoms in young-adult or aged mutant mice, suggesting that the ability to compensate for severe DA deficiency might be higher in the mouse than in humans [25]. Thus, early diagnosis can be instrumental for disease management but this still remains a major hurdle. Currently available treatments provide only symptomatic relief and do not modify the course of the disease [26].

Genetic studies in the past 15 years have identified a number of genetic mutations (e.g. gene duplications/triplications or missense mutations) in certain familial autosomal dominant or recessive monogenic forms of PD (i.e. SNCA, Parkin, PINK1, LRRK2, DJ-1, VPS35, ATP13A2, GBA) [27,28]. Recessively inherited Parkinsonism is frequently associated with early disease onset (before the age of 40 years). Although the majority (>90%) of PD forms are sporadic, the phenotypes of familial and sporadic forms are very similar, implying that they might arise from common underlying mechanisms [3,29]. The study of the function of PD-linked genes has been instrumental to advance our understanding of the cellular processes involved in PD pathogenesis as discussed below. However, most of the genetically engineered mouse models generated through changes in PD-linked gene expression do not exhibit the cardinal feature of progressive loss of mDA neurons in the SNpc [30,31]. To this respect, genetically engineered mice, in which expression of transcription factor genes controlling mDA neuron development are altered, have proven very useful animal models for PD research [11].

2.2. Progressive loss of mDA neurons in En1+/− mice

Previous loss of function studies demonstrated that En1/2 protect mature mDA neurons during late embryonic development against caspase-3-mediated apoptotic cell death in a dose-dependent manner [7–10]. Subsequently, several studies using various mouse models with targeted disruption of *En1/2* alleles showed that En1/2 continues to be required for the survival of adult mDA neurons. The effects of a complete loss of En1 on adult mDA neuron survival could not be analyzed due to neonatal lethality of *En1*−/− mutants (OF1 background) [32]. However, the phenotype of mice heterozygous for En1 *(En1+/−)* has now been extensively characterized by several groups [19,21,22,33,34]. These mice present a normal number of mDA neurons in the SNpc until the age of 6 weeks after birth. After this age, these neurons start to die progressively and their number decreases by about 40% (as compared to wild-type mice) at 48 weeks of age when their number is stabilized. *En1/2* gene dosage effect on survival was also seen in *En1*+/−; *En2*−/− mice (C57BL/6 background) which present a massive loss of mDA neurons in the SNpc of young adult mice. It was shown that En1/2 survival activity on mDA neurons in these mice could be mediated through both, the activation of Erk1/2 MAPK survival pathway and suppression of the pro-apoptotic activity of the pro-neurotrophin receptor p75NTR [10]. Finally, a recent study also analyzed *En1*−/− mice, which are viable on a C57BL/6 background, and reported a much more rapid and pronounced postnatal loss of mDA neurons [35]. All these observations support the idea that En1/2 continues to be required for the survival and/or maintenance of a subset of mDA neurons during adulthood.

It is noteworthy that VTA mDA neurons, located in the vicinity of SNpc, are relatively spared in *En1*+/− mice (20% loss at 48 weeks) as is the case in human PD [36]. These neurons project to the nucleus accumbens, the amygdala, the hippocampus and the prefrontal cortex to form the mesolimbic and mesocortical pathways, involved in motivation, reward, addiction, cognition and memory. The molecular determinants of the selective vulnerability of mDA SNpc neurons as compared to the VTA are still not known. Some of these differences may arise from the fact that SNpc mDA neurons express elevated levels of a highly active glycosylated form of DAT (glyco-DAT) and inwardly rectifying potassium channel (GIRK2), whereas mDA neurons in the VTA express more of the calcium binding protein calbindin D28K (CALB1) [36]. More recent single cell gene expression profiling indicates that there might be a much greater heterogeneity in mDA neurons within the SNpc or VTA [37]. For instance, *En1* expression appears to be higher in mDA neurons located in the lateral ventral part of the SNpc [37], which hosts neurons that preferentially die in *En1*+/− mice; mDA neurons in the ventral tier of the SNpc are also the most vulnerable in human PD.

Loss of SNpc mDA neurons in *En1*+/− mice leads to decreased striatal DA. These mice develop PD-like motor symptoms such as reduced locomotor activity (distance travelled, rearing), increased

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