

● PERSPECTIVE

Limited therapeutic potential of astrocyte elevated gene-1 transduction in an animal model of Parkinson's disease

Biological roles of astrocyte elevated gene-1 (AEG-1): AEG-1, also known as metadherin, was originally identified as a human immunodeficiency virus-1- and tumor necrosis factor-alpha-inducible gene in human fetal astrocytes. The increase in AEG-1 expression is a well-established and important oncogenic event in various types of human cancer, and its upregulation triggers evasion of cellular apoptosis, metastasis, and invasion in cancer (Emdad et al., 2016; Dhiman et al., 2019). AEG-1 can promote tumor progression via multiple phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathways, contributing to an invasive phenotype and angiogenesis (Emdad et al., 2016; Dhiman et al., 2019). In addition, there is evidence for a functional link between AEG-1 and pro-survival mechanisms in various cancers (Dhiman et al., 2019), suggesting that AEG-1 is a key molecule for oncogenic events in cancer.

In central nervous system, astrocytic AEG-1 can regulate the expression of excitatory amino acid transporter 2, and its upregulation can be associated with excitotoxicity, which is related to the dysregulation of glutamate concentration through inhibition of excitatory amino acid transporter 2 expression (Emdad et al., 2016; Yin et al., 2018). On the other hand, astrocytic AEG-1 can be associated with astroglial migration to inhibit the expansion of lesioned area, resulting in neuroprotective effects (Emdad et al., 2016; Yin et al., 2018), suggesting that astrocytic AEG-1 may have ambilaterality in central nervous system. The expression of neuronal AEG-1 is well observed in the adult brain (Yin et al., 2015; Leem et al., 2018). However, there are few reports explaining the role of neuronal AEG-1, unrelated to tumorigenesis and astrocytes, in the adult brain.

Role of AEG-1 in adult dopaminergic (DA) neurons in the substantia nigra: The downregulation of neuronal AEG-1 has recently been shown to reduce the viability of motor neurons in a mouse model of amyotrophic lateral sclerosis by activating apoptotic signaling pathways via inhibition of the PI3K/Akt signaling pathway (Yin et al., 2015). The aberrant activation of apoptotic signaling pathways in the adult brain is a well-known neurotoxic event that is associated with neuronal loss, such as that observed in neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD) (Leem et al., 2018). The PI3K/Akt/mammalian target of rapamycin complex 1 (mTORC1) signaling pathway has been shown to elicit neuroprotective effects on the survival and growth of neurons in the nigrostriatal DA system *in vivo* (Cheng et al., 2011; Leem et al., 2018). However, although maintaining adequate levels of neuronal AEG-1 plays a critical role in neuronal survival in the adult brain, little is known about the neuroprotective role of AEG-1 in PD.

PD, which shows the second-largest number of patients following AD, is one of the representative neurodegenerative diseases. We recently found that the loss of DA neurons in postmortem substantia nigra tissues from patients with PD could be associated with significantly lower levels of AEG-1 expression in nigral DA neurons than that in age-matched controls. In addition, we observed that AEG-1 transduction of nigral DA neurons using adeno-associated virus serotype 1 could protect DA neurons by downregulation of pro-apoptotic molecules, such as cleaved caspase 3 and cleaved poly (ADP-ribose) polymerase 1, in the 6-hydroxydopamine (6-OHDA)-treated animal model of PD (Leem et al., 2018), suggesting that AEG-1 functioned as an anti-apoptotic factor in nigral DA neurons. Furthermore, the decrease in AEG-1 might be involved in the loss of DA neurons, which is one of the key patholog-

ical features of PD. However, the upregulation of AEG-1 in nigral DA neurons was not sufficient to protect the whole nigrostriatal DA projection in the 6-OHDA-treated animal model of PD (Leem et al., 2018).

Cell death mechanisms in PD: Cell death mechanisms are indispensable biochemical reactions including unexpected cell death and the removal of unnecessary cells following an act of cold calculation for survival. Cell death is classically divided into caspase-dependent cell death "Apoptosis" and caspase-independent mechanism "Necrosis" and "Autophagy-induced cell death" (Michel et al., 2016). The aims of these cell death mechanisms are to maintain the cellular homeostasis by regulating the energy balance and cytoplasm pH levels, and eliminating dysfunctional organelles or proteins. However, the occurrence of uncontrollable and excessive cell death, and the failure to eliminate dysfunctional cells can cause various diseases such as cancer, autoimmune disease, and degenerative diseases. PD, which shows neurodegeneration in the nigrostriatal DA system, is also caused by various cell death mechanisms. For these reasons, the control of cell death mechanisms in PD has been recognized as an important strategy to prevent or delay neurodegeneration.

Caspase-dependent DA neuronal loss: Apoptosis, a type of programmed cell death, commonly occurs during development and ageing as an essential mechanism to maintain the homeostasis and cell population in normal tissues, and also triggers immune responses when cells are infected or damaged to restore the physiological conditions by removing abnormal cells (Hartmann et al., 2000). However, the abnormal apoptosis can be associated with neurodegenerative diseases (Hartmann et al., 2000; Kanthasamy et al., 2006; Leem et al., 2018). Moreover, the increased levels of caspase 3 and poly (ADP-ribose) polymerase 1 expression were apparently observed in the substantia nigra of patients with PD (Leem et al., 2018), and downregulation of apoptosis-mediating molecules protected DA neurons in a caspase 3-dependent manner (Hartmann et al., 2000; Kanthasamy et al., 2006; Leem et al., 2018).

Caspase-independent DA neuronal loss: abnormal autophagy-mediated neuronal loss: Autophagy signaling pathway is one of mechanisms to preserve cellular homeostasis by removing the dysfunctional and inessential proteins and organelles, and by suppressing mTORC1-mediated anabolism. Autophagy signaling pathway can be activated by imbalanced energy and deprivation of nutrients, and its unperverted activation can provide a neuroprotective effect by removing the misfolded proteins and dysfunctional organelles (Petibone et al., 2017). In the 6-OHDA- or axotomy-induced animal models of PD, DA degeneration was alleviated by the inhibition of abnormal autophagy signaling following the sustained activation of Akt/mTORC1 signaling pathway (Cheng et al., 2011), suggesting that the perverted dysregulation of autophagy lysosomal pathway could be involved in the pathogenesis shown in PD (Cheng et al., 2011; Leem et al., 2018).

Caspase-independent DA neuronal loss: necroptosis: Necroptosis is another cell death mechanism bearing similar morphological features to necrosis, such as early destruction of membrane integrity, and cell and intracellular organelle swelling. It is initiated by DNA damage and death ligand-triggered signal and regulated in a caspase independent manner (Oñate et al., 2019). The increased activity of mixed lineage kinase domain-like pseudokinase, a necroptosis-mediating marker, was recently observed in the postmortem substantia nigra of patients with PD. In addition, 6-OHDA-treated animal model of PD also showed necroptosis, particularly associated with DA axonal degeneration (Oñate et al., 2019), suggesting that the control of necroptosis might be important for DA axonal protection against PD.

Perspective for enhancement of AEG-1-induced therapeutic potential against PD: We recently reported that the expression of

AEG-1 was significantly decreased in damaged DA neurons in the substantia nigra of patients with PD and 6-OHDA-treated mice compared to age-matched controls and normal mice, respectively. However, no significant reductions in the AEG-1 in the hippocampi of patients with AD was noted (Leem et al., 2018), suggesting that the decreased level of AEG-1 was a specific event that occurred in damaged DA neurons, and that AEG-1 downregulation and the loss of nigral DA neurons in PD might be clinically correlated. In addition, we showed that increasing the expression of AEG-1 using adeno-associated virus attenuated the apoptotic death of nigral DA neurons caused by 6-OHDA administration in mice (Leem et al., 2018), which might be critical for intervening in the pathogenesis of neurodegenerative diseases and developing therapies that prevent the loss of DA neurons in PD.

However, the overexpression of AEG-1 in DA neurons was not sufficient to protect the whole nigrostriatal DA projection in the animal model of PD owing to its limited protective effects, as it did not affect the Akt/mTORC1 activity, and aberrant accumulation of autophagic components following 6-OHDA administration (Leem et al., 2018), which could also contribute to the neurotoxic effects on the nigrostriatal DA system (Cheng et al., 2011). To overcome this limitation of AEG-1, we examined whether further transduction of the constitutively active ras homolog enriched in brain [Rheb(S16H)] as an intracellular Akt/mTORC1 activator, which inhibited neurotoxicity by abnormal autophagic regulation in animal models of PD (Cheng et al., 2011), into AEG-1-overexpressing DA neurons could induce better protective effects on the whole nigrostriatal DA system in the animal model of PD (Leem et al., 2018). We observed that the synergistic effects of the two factors could restore the nigrostriatal DA system that was disrupted by 6-OHDA administration. These effects were more obvious in the presence of AEG-1 than in its absence, even more than Rheb(S16H) alone (Leem et al., 2018).

Although the induction of AEG-1 alone was not sufficient to preserve the nigrostriatal DA projections in the animal model of PD, these results suggest that AEG-1 may be an important anti-apoptotic factor against the loss of DA neurons in PD, and its sustained levels in nigral DA neurons may potentiate the therapeutic effects of treatment approaches such as Rheb(S16H) administration, on degeneration of the DA pathway that is characteristic of PD (Figure 1). Thus, the development of additional treatment or enhanced constitutively active AEG-1 with enhanced protective activity, resulting in inhibition of both apoptotic and non-apoptotic cell death, are necessary for therapeutic effects against PD.

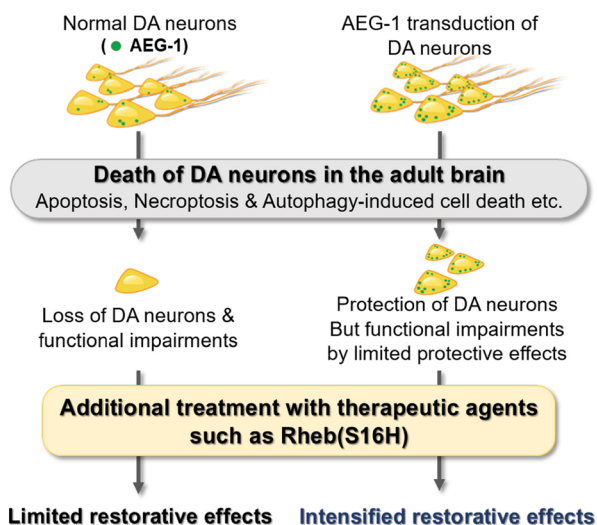


Figure 1 Schematic of the potential therapeutic effects following AEG-1 transduction of nigral DA neurons in the adult brain. AEG-1: Astrocyte elevated gene-1; DA: dopaminergic.

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