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PERSPECTIVE

Releasing Nrf2 to promote neurite outgrowth

Roles of Keap1-Nrf2 pathway in brain: Neuronal survival and neurogenesis are impaired in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Winner et al., 2011). Genetic up-regulation of growth factors enhanced neuronal survival and neurogenesis, improved neuronal functions and halted disease progression in animal models of Alzheimer's disease (Jin et al., 2004). Pharmacological stimulation of neurogenesis also holds promise in the therapy of neurodegenerative diseases (Abdipranoto et al., 2008). Different neurotrophic factors have been evaluated for promoting neuronal survival and stimulating neurogenesis towards functional recovery (Hennigan et al., 2007; Allen et al., 2013). Nerve growth factor (NGF) is a typical neurotrophic factor in the regulation of neuronal survival, growth and differentiation. However, the clinical application of NGF is restricted by delivery issues and adverse effects (Spedding and Gressens, 2008; Allen et al., 2013). As an alternative approach, small molecules that mimic or enhance the effect of neurotrophic factors become promising drug candidates for treating neurodegenerative disorders (Price et al., 2007). Therefore, it is important to understand the molecular mechanisms by which small molecules could exhibit neurotrophin-like neuroprotective and neurogenic activities. In fact, nuclear factor erythroid 2-related factor 2 (Nrf2) is an important endogenous redox-sensitive transcription factor. Under normal conditions, Nrf2 mainly resides in the cytosol in complex with Kelch-like ECH-associated protein 1 (Keap1). Upon stimulation, Keap1-Nrf2 complex is dissociated from each other, leading to the nuclear translocation of Nrf2 and induction of various phase II defense enzymes, antioxidant proteins and anti-inflammatory factors (Ma, 2013). Recent studies have consolidated the importance of Nrf2 in the neuroprotection and neurogenesis (Zhao et al., 2009; Wakabayashi et al., 2010; Kärkkäinen et al., 2014). Therefore, Nrf2 is a promising therapeutic target for treating neurological diseases.

Natural products for neuroprotection and neurogenesis: Natural products represent a rich resource for the development of new drugs for the protection of neurons against oxidative insults and the promotion of neurogenesis (Ho et al., 2012). Recent studies demonstrated that curcumin, Z-ligustilide and other types of polyphenols could elicit neuroprotective and neuritogenic activities in *in vitro* and *in vivo* models of neurodegenerative diseases (Ataie et al., 2010; Scapagnini et al., 2011; Joshi and Johnson, 2012; Qi et al., 2012). Some small molecules constitute a class of electrophilic drugs that could directly modify Keap1 and subsequently activate Nrf2 pathway as a key neuroprotective and neuritogenic mechanism (Scapagnini et al., 2011; Joshi and Johnson, 2012). In fact, Keap1 is a cysteine rich protein for sensing the intracellular oxidative stressors including electrophiles. Several key cysteine residues such as C257, C273, C288, and C297 in Keap1 could react with electrophilic compounds (Zhang, 2006; Wahlang et al., 2015). Importantly, electrophilic modification of Keap1 consequently releases Nrf2 for nuclear translocation and induction of various neuroprotective genes (Eggler et al., 2005; Ma, 2013). On the other hand, caffeic acid and its derivatives were mainly evaluated for antioxidant, anticancer, anti-inflammatory, anti-human immunodeficiency virus (HIV) and neuroprotective activities (Tolba et al., 2013; Shi et al., 2014; Silva et al., 2014; Yang et al., 2014a, b). Little is known about the activities of caffeic acid derivatives in neurogenesis and the underlying mechanism.

Effect of caffeic acid derivatives on neuronal survival and neurite outgrowth: We recently synthesized a novel caffeic acid derivative N-propargyl caffeate amide (PACA) and investigated its neuroprotective and neuritogenic activities. We found that PACA not only attenuated 6-hydroxydopamine (6-OHDA) neurotoxicity but also potentiated NGF-induced neurite outgrowth in dopaminergic PC12 cells and primary rat midbrain neurons (Yang et al., 2015). To further elucidate the molecular mechanisms underlying the neuroprotective and neuritogenic activities of PACA, we focused on the identification of the PACA-modified proteins. We took the advantage of the alkyne group in PACA structure for its reactivity toward Azido group *via* Click chemistry Azide-Alkyne cycloaddition (Gordon et al., 2012; McKay and Finn, 2014). After affinity isolation, Keap1 was identified as a predominant PACA-modified protein (Yang et al., 2015). We subsequently demonstrated that PACA induced the nuclear translocation of Nrf2 and the expression of antioxidant heme oxygenase-1 (HO-1) *via* direct modulation of Keap1. HO-1 is a key Nrf2 target gene and catalyzes the degradation of pro-oxidant heme into antioxidant, anti-inflammatory biliverdin/bilirubin, carbon monoxide and ferrous ion (Motterlini and Foresti, 2014). After the treatments with PACA and HO-1 specific inhibitor tin protoporphyrin IX (SnPP), alone or in combination, we examined the effect of 6-OHDA on the neuronal survival and the neurite outgrowth in PC12 cells. Our results suggest that PACA enhanced the cellular resistance against 6-OHDA toxicity and potentiated NGF-induced neurite outgrowth *via* inducing HO-1 expression. As shown in **Figure 1**, chemically, PACA bears catechol structure. We speculate that PACA may be oxidized into *o*-quinone structure by the intracellular oxidases. It is well-known that *o*-quinones are reactive towards the cysteine residues in Keap1 (Zhang, 2006; Wahlang et al., 2015). Covalent modification of Keaps1 disrupts the Keap1-Nrf2 complex. Nrf2 is thereby released for nuclear translocation and subsequent induction of HO-1 expression. As HO-1 inhibitor SnPP diminished the effect of PACA on neuronal survival and neurite outgrowth, we concluded that PACA exhibited dual neuroprotective and neuritogenic effects *via* direct modification of Keap1 and subsequent release of Nrf2.

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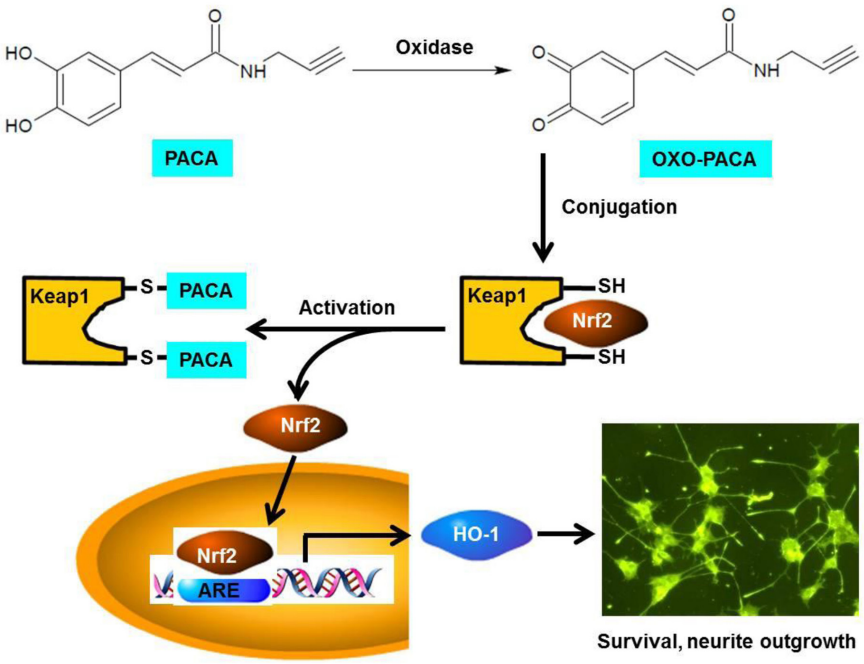

Figure 1 Potential mechanisms underlying the neuroprotective and neuritogenic effects of PACA.
PACA is oxidized into chemically reactive *o*-quinone. PACA-derived *o*-quinone sequentially modifies Keap1, activates Nrf2 and induces HO-1 to promote neurite outgrowth. HO-1: Heme oxygenase-1; Keap1: Kelch-like ECH-associated protein 1; Nrf2: nuclear factor erythroid 2-related factor 2; PACA: N-propargyl caffeate amide.

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