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Interleukin-18 increases expression of kinases involved in tau phosphorylation in SH-SY5Y neuroblastoma cells

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ABSTRACT

Inflammatory cytokines, produced mainly by activated microglia in the brain, can enhance neuronal degeneration and the amyloid-β-plaque production involved in Alzheimer's disease (AD). We previously demonstrated that the expression of the pro-inflammatory cytokine interleukin-18 (IL-18) colocalizes with plaques and hyperphoshorylated tau containing neurons in AD patients. Here we exposed neuron-like, differentiated SH-SY5Y neuroblastomas to IL-18 and observed that the protein levels of p35, Cdk5, GSK-3β, and Ser15-phosphorylated p53 increased during 6 h-24 h. Tau phosphorylation and expression of cyclin G1, involved in neuronal regeneration, increased at 72 h. In vivo, over-expression of IL-18 may induce hyperphosphorylation of tau and induce cell cycle activators.

1. Introduction

Neurofibrillary changes, in the form of amyloid-β composed neuritic plaques, neuropil threads, and neurofibrillary tangles (NFTs), are key histological features of Alzheimer's disease (AD) (Khachaturian, 1985; Mirra et al., 1991; Braak and Braak, 1991). The main components of NFTs in AD are the paired helical filaments (PHF) of abnormally phosphorylated tau-protein. In addition, over-expression of inflammatory cytokines IL-1 and IL-6 has been consistently shown in the AD brain (Vandenabeele and Fiers, 1991; Cacabelos et al., 1994; Griffin et al., 1998; Bauer et al., 1991; Hampel et al., 1999; Lu and Wood, 1993). We have shown also that expression of a potent upstream cytokine IL-18 (interferon-γ-inducing factor, IL-1γ) is increased in AD brain (Ojala et al., in press). IL-18 shares structural similarities with the IL-1 family of proteins, e.g. it can enhance production of toxic inflammatory molecules such as IFN-γ (Okamura et al., 1995) and IL-1β (Joosten et al., 2003). Over-expression of inflammatory molecules such as IL-1β has many detrimental effects in the brain, e.g. it contributes to the impairment of cognitive abilities (Holmes et al., 2003; Tarkowski et al., 2003) and to the generation of neuritic plaques (Chang et al., 1999; Lahiri et al., 2003). IL-1β can also induce an increase in tau phosphorylation and tangle formation, which seems to be mediated partially by MAPK p38 (Li et al., 2003). IL-6 is also known to be an activator of the MAPK-p38 signaling pathway (Quintanilla et al., 2004). Nevertheless, IL-1β, IL-6 and TNF-α may also function as regulating factors affecting neuronal and central nervous system (CNS) development (Zhao and Schwartz, 1998; Engele, 1998; Doherty, 2007), although their exact role and direct or indirect effects in the development and also in the functioning of the nervous system are still poorly understood.

Neuronal loss and severity of dementia correlate better with the amount of NFTs rather than with amyloid pathology in AD (Gomez-Isla et al., 1997). Tau, one of the microtubule-associated proteins (MAPs), is predominantly expressed in neurons, and further, it is detected mainly in the axons of mature neurons. Correct phosphorylation status of tau is associated with its normal functioning, for instance it is crucial in determining neuronal polarity (Mandell and Banker, 1996; Burack and Halpain, 1996; Johnson and Stoothoff, 2004). Tau can be abnormally phosphorylated or be post-translationally modified in other ways in more than 20 neurodegenerative disorders, which form the disease group called tauopathies. All these tauopathies, including AD, are characterized by the presence of intracellular inclusions formed from filamentous tau-proteins (reviewed Lee et al., 2001; Hernández and Avila, 2007). In AD, for unknown reasons, tau can become excessively phosphorylated by several kinases, which leads to microtubule disassembly. Hyperphosphorylated tau relocalizes from axonal to somatodendritic compartments where it forms PHF and subsequently intracellular NFTs. The neurofibrillary pathology, including PHF,

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dystrophic neurites, and NFTs, evokes a loss of axonal integrity and eventually, a decline in connectivity and synapses (reviewed Morishima-Kawashima et al., 1995; Khatoon et al., 1994; Johnson and Stoothoff, 2004; Stoothoff and Johnson, 2005; Hernández and Avila, 2007).

Two important kinases involved in phosphorylating tau, either normally or abnormally, are glycogen synthase kinase GSK-3β and cyclin dependent kinase 5 (Cdk5), which are both serine/threonine, proline-directed kinases (Johnson and Stoothoff, 2004). Both kinases are involved in AD but also in the development of neurons (Ohshima et al., 1996; Johnson and Stoothoff, 2004; Naska et al., 2006; Nguyen et al., 2002; Hooper et al., 2008). GSK-3β (46 kD) is involved in a diverse array of signaling pathways, including those implicated in neuronal polarity (Jope et al., 2007; Harwood, 2001; Jiang et al., 2005), regulation of neuronal lamination (Beffert et al., 2002), regulation of cell adhesion, inflammation and tau exon 10 splicing (Jope et al., 2007; Hernández et al., 2004). GSK-3β activity is modulated by insulin and Wnt signaling and both pathways act in a negative regulatory manner (Hooper et al., 2008). Cdk5 (31 kDa) is required for a proper development of the CNS, and possibly also in neuronal differentiation by affecting cytoskeleton structure and organization (Ohshima et al., 1996). Both Cdk5 and its activator p35 are enriched in the processes and growth cones of neurons, and seem to be required for neurite growth (Nikolic et al., 1996; Patrick et al., 1999). However, inflammation may increase Cdk5 activity due to increased calpain activity (Pareek et al., 2006), which cleaves p35 to more stable p25. The accumulation of p25 correlates with an increase in Cdk5 kinase activity, and Cdk5/p25 complex can hyperphosphorylate tau. Thus, p25 accumulation may precede the formation of NFT in the AD brain (Patrick et al., 1999). Cdk5 is also involved in regulating pain signaling caused by inflammation (Pareek et al., 2006), but activity of this kinase seems to have a role also in neuronal survival (Patrick et al., 1999).

Degenerating neuronal cells in AD have been shown to exhibit phenotypic changes that are characteristic to cells re-entering to the cell division cycle (McShea et al., 2007). The role of the inflammatory cytokines in this re-entry is not understood, but it has been suggested that tau hyperphosphorylation may also induce abnormal, incomplete neuronal cell cycle re-entry (Andorfer et al., 2005). Generally, the changes include activation of related signal transduction pathways and cell cycle-dependent kinases as well as transcriptional activation, which can lead to cytoskeletal alterations and DNA replication (Yang et al., 2001; McShea et al., 2007; Nagy, 2000). The hypothesis, that some degenerating or sublethally damaged neurons may re-enter the cell cycle possibly as an effort to recover (Fig. 1), is supported by the finding that the expression of G/S phase regulating cyclin G1 (CG1) in association with cyclin dependent kinases, Cdk2 and Cdk5, increases significantly in hippocampi of patients suffering from mild cognitive impairment (MCI) (Sultana and Butterfield, 2007).

Our previous results from the post-mortem brain samples of AD patients suggested that the protein level of IL-18 was associated with the tau-protein levels, and that was apparent in immunohistochemical level as well as in CSF (Ojala et al., in press). Therefore, in this study, we wanted to examine the impact of IL-18 on living, one-cell type neuronal culture, and we selected as neuron-like differentiated human SH-SY5Y neuroblastoma cells. Our targets were in AD, but also in development of neurons involved Cdk5, p35 and GSK-3β, and their target tau and its phosphorylation. Further, since expression of CG1 has been shown to associate with Cdk5 and possibly nerve regeneration (Morita et al., 1996), we examined whether IL-18 has any impact on cell cycle associated proteins.

2. Materials and methods

2.1. Cell culture and IL-18 treatment

SH-SY5Y neuroblastomas (DSMZ; Braunschweig, Germany) were cultured in Dulbeccos's medium (BioWhittaker/Cambrex; Verviers, Belgium) containing 4.5 g/l glucose, 5% fetal bovine serum (HyClone/Pierce; Logan, UT, USA), 2 mM L-glutamine (Cambrex), 100 U/ml penicillin and 10 μg/ml streptomycin (Cambrex). The cells were plated as 10^5 cells/well into the 12-well plate (Nunc™; Roskilde, Denmark), and differentiated with three day 10 μM all-trans retinoic acid (ATRA; Sigma-Aldrich; St. Louis, MO, USA) treatment followed by four day 50 ng/ml human recombinant BDNF (rchBDNF; Alomone labs; Jerusalem, Israel) treatment.

Recombinant human IL-18 (rchIL-18; MBL Medical Biological laboratories Co, Ltd.; Naka-ku Nagoya, Japan) was added to culture medium as 40–160 ng/ml for 6, 24, 48, or 72 h. RchBDNF was present

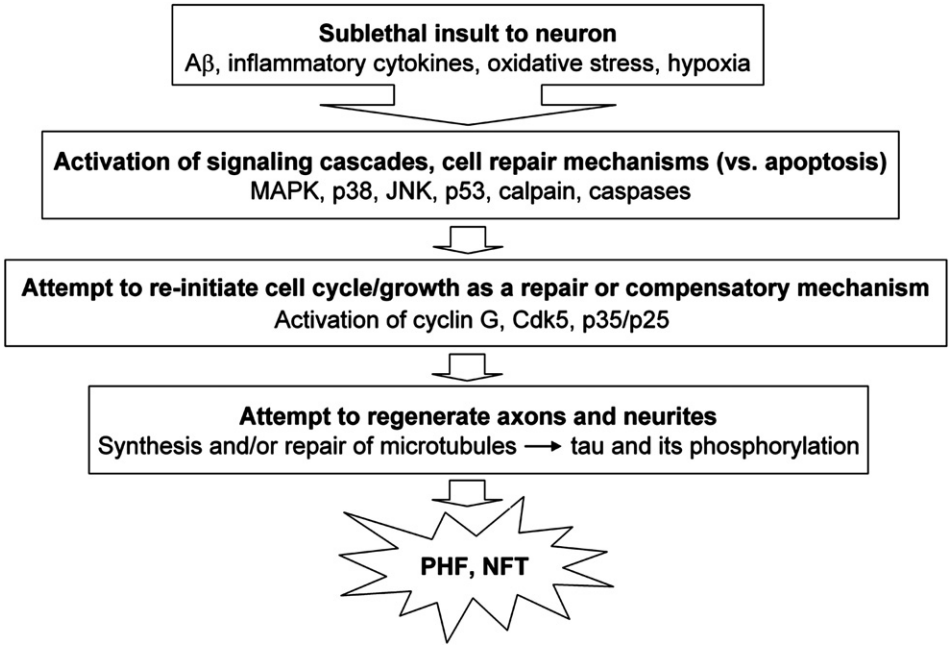

Fig. 1. Sublethal damage in a neuron likely may well induce its repair mechanisms, which can lead to activation of the kinases involved in cell cycle and phosphorylation of tau.