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Polo-like kinase 3 (PLK3) mediates the clearance of the accumulated PrP mutants transiently expressed in cultured cells and pathogenic PrP^{Sc} in prion infected cell line *via* protein interaction

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ABSTRACT

Polo-like kinases (PLKs) family has long been known to be critical for cell cycle and recent studies have pointed to new dimensions of PLKs function in the nervous system. Our previous study has verified that the levels of PLK3 in the brain are severely downregulated in prion-related diseases. However, the associations of PLKs with prion protein remain unclear. In the present study, we confirmed that PrP protein constitutively interacts with PLK3 as determined by both *in vitro* and *in vivo* assays. Both the kinase domain and polo-box domain of PLK3 were proved to bind PrP proteins expressed in mammalian cell lines. Overexpression of PLK3 did not affect the level of wild-type PrP, but significantly decreased the levels of the mutated PrPs in cultured cells. The kinase domain appeared to be responsible for the clearance of abnormally aggregated PrPs, but this function seemed to be independent of its kinase activity. RNA-mediated knockdown of PLK3 obviously aggravated the accumulation of cytosolic PrPs. Moreover, PLK3 overexpression in a scrapie infected cell line caused notable reduce of PrP^{Sc} level in a dose-dependent manner, but had minimal effect on the expression of PrP^{C} in its normal partner cell line. Our findings here confirmed the molecular interaction between PLK3 and PrP and outlined the regulatory activity of PLK3 on the degradation of abnormal PrPs, even its pathogenic isoform PrP^{Sc}. We, therefore, assume that the recovery of PLK3 in the early stage of prion infection may be helpful to prevent the toxic accumulation of PrP^{Sc} in the brain tissues.

Abbreviations: PLKs, polo-like kinases; KD, kinase domain; PBD, polo-box domain; TSE, transmissible spongiform encephalopathies; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Straüssler–Scheinker; FFI, fatal familial insomnia; BSE, bovine spongiform encephalopathy; AD, Alzheimer's disease; CNS, central nervous system; FL, full-length; PG5-PrP, wild-type human PrP; CYTO-PrP, a human PrP mutant lacking the signal peptide and GPI anchor; PG14-PrP, a human PrP mutant with nine extra octarepeats insertion associated with genetic CJD; SMB–PS, SMB cell line with pentosan sulfate cure it; IP, immunoprecipitation.

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1. Introduction

Transmissible spongiform encephalopathies (TSE) are transmissible, lethal neurodegenerative disorders, including Creutzfeldt–Jakob disease (CJD), kuru, Gerstmann–Straüssler–Scheinker (GSS) syndrome and fatal familial insomnia (FFI) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle (Haik and Brandel, 2014; Mastrianni, 1998). The progressive disorders affect the central nervous system leading to memory loss, personality changes, ataxia, and neurodegeneration. Histologically, the hallmarks of TSE are neuronal loss, vacuolation, and reactive gliosis. A central event in TSE pathogenesis is the conformational conversion of PrP^{C}, a normal cell-surface glycoprotein into an abnormal isoform PrP^{Sc} (Stahl et al., 1993). It is thought that such abnormally folded aggregates or more probably a metastable conversion intermediate causing infectivity and neurodegeneration (Weissmann, 1991).

PLK3 belongs to the polo-like kinase family, which has an extensive tissue distribution, whereas PLK1 is specifically found in proliferation tissues. PLKs share a conserved sequence motif characterized by two regions: a highly conserved N-terminal serine/threonine catalytic domain (KD) and a C-terminal non-catalytic termed the polo-box domain (PBD). PBD participates in regulating substrate interactions, subcellular localization and autoinhibition of the PLKs (de Carcer et al., 2011; Lowery et al., 2005). Different PLK subfamilies have distinct functions and the expression is regulated differentially in cells and tissues in response to stimuli (Winkles and Alberts, 2005). PLK3 has been reported to primarily take part in regulating microtubule dynamics, centrosomal functions, cellular adhesion and DNA damage responses (Bahassi el, 2011). In addition to the well-established roles in cell cycle, converging lines of evidence has implied that PLK3 is also constitutively expressed in post-mitotic neurons and partakes in synaptic plasticity in neuronal dendritic spines through the phosphorylation of the spindle-associated protein SPAR (Seeburg et al., 2005). Recent literature suggests that PLKs have an affiliation with neuronal aggregation in neurodegenerative diseases. Among them, PLK2 interacts with a neuronal phosphoprotein, α-synuclein, the latter accumulates in several neurological diseases, such as Parkinson's and dementia with Lewy bodies (Oueslati et al., 2013). The expression level of PLK1 and activity are highly enhanced in susceptible hippocampal and cortical phospho tau-positive neurons of Alzheimer disease (AD) patients (Harris et al., 2000; Song et al., 2011). The abnormal alterations of PLKs in prion diseases have also been documented (Wang et al., 2013). Our earlier research has found that the expression levels of PLK3 substantially decrease whilst PLK1 increase in brains of scrapie-infected hamsters, leading to dysfunctions of a range of biological indicators associated with cell cycle. However, it remains unsettled about the molecular recognition between PLK3 and prion proteins and the subsequently biological significances.

In the present study, we observed that the endogenous PLK3 and PLK1 were able to interact with PrP in both normal and scrapie-infected hamsters' brain tissues. The molecular interaction between PLK3 and PrP proteins were further verified by recombinant proteins expressed in cultured mammalian cells and in *Escherichia coli in vitro*. Intriguingly, the overexpression of PLK3 did not affect the expression level of wild-type PrP, but significantly diminished the levels of the mutated PrPs transiently expressed in cultured cells. The propensity of PLK3 to clear the abnormally aggregated PrPs appeared to be determined by the KD region, but seemed to be independent of its kinase activity. Suppression of endogenous PLK3 expression by RNAi exacerbated the levels of the expressed mutated PrPs in the cells. Moreover, an overexpression of PLK3 in a prion-infected cell line SMB-S15 caused drastically decline of PrP^{Sc} in a dose-dependent manner. Taken together, our results implicate that PLK3 may tightly regulate the degradation of abnormal PrPs, which will be an attractive and viable therapeutic avenue for prion-related disorders.

2. Materials and methods

2.1. Preparations of brain homogenates

Four-week old Chinese golden hamsters inoculated intracerebrally with 2 μl of 10% brain homogenates generated from hamster-adapted scrapie agent 263K-infected hamsters at the site that above the superciliary arch with a depth of 4–5 mm (Shi et al., 2012; Yao et al., 2005). The normal control hamsters of the same age were prepared by the administration of equal amount of physiological saline with the same method. The brain samples of the agent 263K-infected hamsters collected at terminal stage of the disease were described elsewhere (Gao et al., 2004; Zhang et al., 2004). Stored brain samples from scrapie-infected hamsters, as well as from normal hamsters and C57 BL/6 (C57) mice, were homogenized in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% $C_{24}H_{40}O_4$·Na in 10 mM Tris-HCl, pH 7.4) containing Cocktail Set III (Merck, 539134). The homogenates were centrifuged at 2000 × *g* for 10 min, and the supernatant fractions were collected and frozen at −80 °C for further tests. Usage of animal specimens in this study was approved by the Ethical Committee of the National Institute for Viral Disease Prevention and Control, China CDC under protocol 2009ZX10004-101. Animal housing and experimental protocols were in accordance with the Chinese Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Plasmids

Recombinant plasmids expressing human PG5-PrP (wild-type human PrP), PG14-PrP (a human PrP mutant with nine extra octarepeats insertion associated with genetic CJD), and CYTO-PrP (a human PrP mutant lacking the signal peptide and GPI anchor) were previously generated based on the vector pcDNA3.1 (Wang et al., 2011b). Plasmids pEGFP–PLK3–FL (expressing the full-length PLK3 from 1 to 646 aa), pEGFP–Plk3–PBD (expressing the PBD region of PLK3 from 312 to 646), pEGFP–PLK3–KD (expressing the KD region of PLK3 from 1 to 334) were received as kind gifts from Dr. Wei Dai (Yang et al., 2008). Plasmids pEGFP–PLK3–PBD (23267) and pEGFP–PLK3–T219D (23268) were purchased from Addgene. Those recombinant plasmids for PLK3 were constructed based on a mammalian expressing vector pEGFP-N1, which will produce a fusion protein with an EGFP tag at the C-terminus.

2.3. Antibodies and reagents

The specific primary antibodies used in this study included anti-PLK1 (Abcam, ab17057), anti-PLK3 (Cell Signaling Technology, #4896), anti-PrP monoclonal antibody (mAb) 3F4 (Chemicon, MAB1562-K), anti-PrP mAb 6D11 (Santa Cruz, sc-58581), anti-GFP (Abcam, ab290), anti-β-actin (Santa Cruz, sc-4778). Horseradish peroxidase (HRP)-conjugated secondary antibodies (115-035-003 and 111-035-003) were from Jackson ImmunoResearch Labs. Alexa Fluor probe labeled secondary antibodies (A-11034 and A-11004), DAPI (D3571), Dynabeads Protein G (10007D), and Lipofectamine 2000 transfection reagent (11668019) were from Life Technologies. Protease inhibitor Cocktail set III (539134) and Proteinase K (124568) were from Merck. Enhanced chemoluminescence (ECL) system (NEL103E001EA) was from PerkinElmer.