Neuroscience Letters 583 (2014) 92–97

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Microglial p53 activation is detrimental to neuronal synapses during activation-induced inflammation: Implications for neurodegeneration

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HIGHLIGHTS

- Activation-induced microglial p53 is transcription-dependent.
- Inhibiting microglial p53 rescues neuronal synaptic marker loss and cell death.
- Selective depletion of microglia from mixed cultures abolishes early changes to neuronal synapses.

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 27 August 2014
Accepted 28 August 2014
Available online 7 September 2014

Keywords:
P53
Microglia
Synapse
Neurodegeneration

ABSTRACT

P53 is a tumour suppressor protein thought to be primarily involved in cancer biology, but recent evidence suggests it may also coordinate novel functions in the CNS, including mediation of pathways underlying neurodegenerative disease. In microglia, the resident immune cells of the brain, p53 activity can promote an activation-induced pro-inflammatory phenotype Jayadev et al. (2011) [1], as well as neurodegeneration Davenport et al. (2010) [2]. Synapse degeneration is one of the earliest pathological events in many chronic neurodegenerative diseases Conforti et al. (2007) and Clare et al. (2010) [3,4] and may be influenced by early microglial responses. Here we examined synaptic properties of neurons following modulation of p53 activity in rat microglia exposed to inflammatory stimuli. A significant reduction in the expression of the neuronal synaptic markers synaptophysin and drebrin, occurred following microglial activation and was seen prior to any visible signs of neuronal cell death, including neuronal cleaved caspase-3 activation. This synaptic marker loss together with microglial secretion of the inflammatory cytokines tumour necrosis factor α (TNF-α) and interleukin 1-β (IL-1β) was abolished by the removal of microglia or inhibition of microglial p53 activation. These results suggest that transcriptional-dependent p53 activities in microglia may drive a non-cell autonomous process of synaptic degeneration in neurons during neuroinflammatory degenerative diseases.

1. Introduction

P53, a tumour suppressor gene encoding a 393 amino acid, 53 kilo Dalton (kDa) protein product, belongs to a family of highly homologous proteins [5,6] commanding crucial and multi-faceted functions in cell-cycle control, apoptosis, and maintenance of genetic stability [7]. In microglia, the resident immunocompetent cells of the CNS, p53 mediates microglial activation and the subsequent pro-inflammatory phenotypes seen in many neurodegenerative diseases [1,2,8,9], but the mechanisms of p53-driven microglial-evoked neurotoxicity remain unclear.

Degeneration of synapses is an early pathological event in many chronic neurodegenerative diseases [3,5] and activation of toll like receptors (TLR) is implicated in innate immunity and neuroinflammatory processes within the CNS [10]. Moreover, p53 activity has recently been linked to TLR signalling in diverse mammalian cell types [11,12]. To determine whether microglial p53 activation influences neuronal synapse integrity, we measured the protein expression levels of two key synaptic proteins; synaptophysin, and drebrin in the presence of TLR-stimulated stimulated microglia or following exposure to microglial conditioned medium (MGCM).

Abbreviations: PFTα, pifithrin-α; PFTμ, pifithrin-μ; iNOS, inducible nitric oxide synthase; IL-1β, interleukin 1β; TLR, toll-like receptor; TNF-α, tumour necrosis factor α; LME, leucine-methyl-ester; LPS, lipopolysaccharide; PI, propidium iodide; DAPI, 4′-6-diamidino-2-phenylindole-2HCl; MEM, minimum essential medium with Earle's salts.

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http://dx.doi.org/10.1016/j.neulet.2014.08.049
0304-3940/

Incubation of neurons with lipopolysaccharide (LPS)-stimulated MGCM or by direct activation of microglia in the neuronal–glial cultures resulted in reduced expression of synaptophysin and drebrin, prior to the triggering of neuronal death cascades.

These effects were driven by the specific microglial activation of p53 and correlated with increased phosphorylated-p53 expression in microglia. Selective elimination of microglia abolished the disruption of neuronal synaptic marker expression and microglial p53 inhibition abolished inflammatory cytokine production. Overall, these results indicate that transcriptional-dependent p53 activities in microglia may be responsible for a non-cell autonomous process of synaptic degeneration in neurons during inflammation.

2. Materials and methods

2.1. Cell culture preparation and treatment

Primary cultured rat microglia and cerebellar granule cell neuronal/glial (CGCs) cultures were prepared from 5-day-old Sprague Dawley rats as previously described [13], in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986. Where indicated, microglia or CGCs were treated directly with pifithrin-α (PFTα) (Sigma, P4359) (10 μM), pifithrin-μ (PFTμ) (Sigma, P0122) (5 μM), and lipopolysaccharide (LPS, 1 μg/ml (Sigma) for 24 or 48 h with cells treated with PFTα or PFTμ 1 h before treatment with LPS. Microglial conditioned medium (MGCM) was collected and aliquots snap-frozen until required. MGCM was subsequently used at a 1:1 ratio with CGC medium.

2.2. Specific depletion of microglia from neuronal cultures

Microglia in CGC cultures were removed by treatment with 25 mM leucine-methyl-ester (LME), as previously described [14,15]. Briefly, cells were exposed to LME for 1 h, followed by washing, replacement of original medium, and resting for 24 h before further treatment. Control cultures were treated identically except for the omission of LME.

2.3. Assessment of apoptosis and cell death in neuronal cultures

Apoptosis and cell death were quantified on live, non-fixed CGCs to determine the effects of MGCM on neuronal survival after 24 or

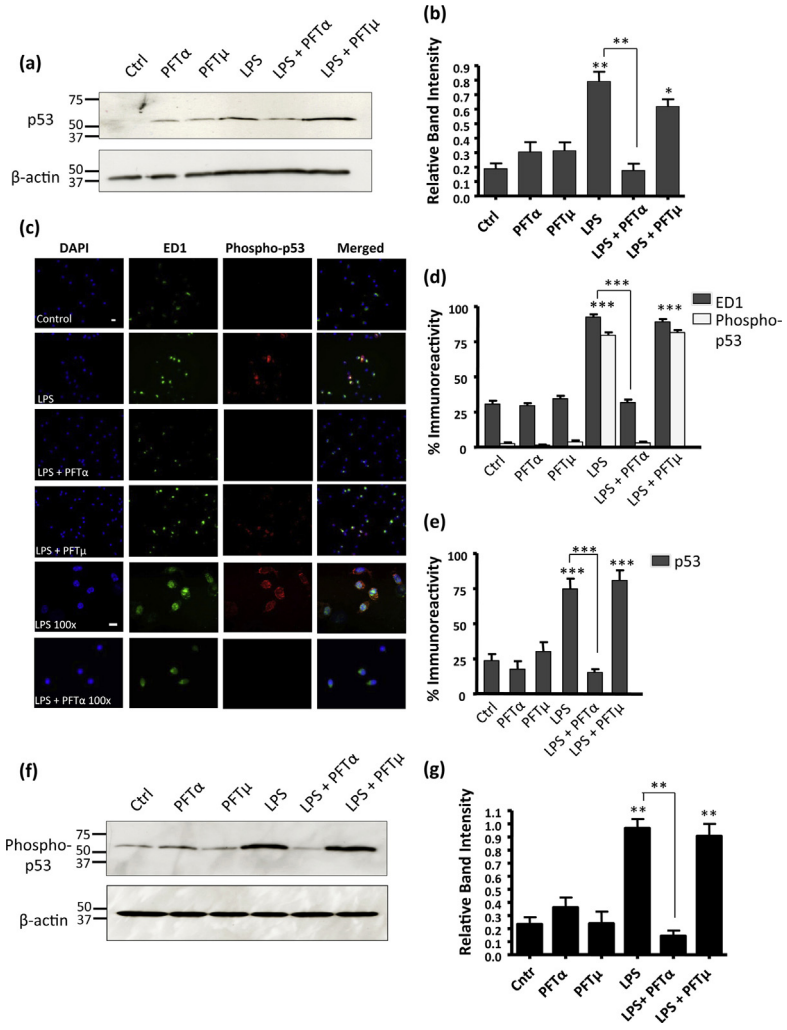

Fig. 1. Expression of microglial p53 and phosphorylated-p53 induced by LPS is inhibited by pifithrin-α. Representative western blot (a) of p53 expression in primary rat microglia after 24 h exposure to pifithrin-α (PFTα, 10 μM), pifithrin-μ (PFTμ, 5 μM), LPS (1 μg/ml), LPS + PFTα, LPS + PFTμ. (b) Densitometry of p53 expression from 3 western blots for primary microglia treated as in (a) normalised to β-actin expression. (c) Immunoreactivity of phosphorylated-p53 (Phospho-p53, red), ED1 (green) with DAPI counterstain (blue) in primary microglia treated as in (a). Magnification ×40 and ×100. Scale bar 10 μm. Quantification of microglial cells staining positive for Phospho-p53 and ED1 (d). (e) Quantification of microglial cells staining positive for p53 alone. (f) Representative western blot of phospho-p53 expression in primary rat microglia treated as in (a). (g) Densitometry of phospho-p53 expression from 3 western blots for primary microglia treated as in (f) normalised to β-actin expression. Data in all bar charts represent mean ± SEM, significance levels compared with control, or as indicated by connecting lines, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)