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Smurf1 regulates macrophage proliferation, apoptosis and migration via JNK and p38 MAPK signaling pathways

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ABSTRACT

Smad ubiquitylation regulatory factor 1 (Smurf1) has been identified to play a critical role in bone homeostasis, development, cell cycle regulation and tumorigenesis. However, the role of Smurf1 in macrophage proliferation, apoptosis and migration is still unclear. Here, we show that Smurf1 expression was elevated in LPS-induced RAW264.7 macrophage and mouse embryonic fibroblasts (MEFs). And we found that knockdown of Smurf1 suppresses macrophage proliferation but promotes apoptosis and migration. Furthermore, JNK and p38 MAPK signaling were upregulated in Smurf1-depleted cells. And inhibition of JNK and p38 MAPK signaling in Smurf1 knockdown cells rescue the phenotypes of macrophage proliferation, apoptosis and migration. Therefore, our study suggests that Smurf1 is a new positive regulator for macrophage proliferation and apoptosis, but a negative regulator for macrophage migration.

1. Introduction

In the haematopoietic system, macrophages, which are ancient cells in metazoan phylogeny and are found in all tissues in adult mammals, are the most plastic cells (Wynn et al., 2013). It is proposed that the differentiation of macrophage is an integral part of organogenesis. Premacrophages (pMacs) initiate their macrophage program by multiple transcriptional regulators expression and macrophages become tissue-specific. Tissue-resident macrophages include epidermal Langerhans cells, liver Kupffer cells, lung alveolar cells and brain microglia (Mass et al., 2016). Macrophages present key cellular components in innate immune system and have roles in immune regulation, development, tissue remodeling and phagocytosis for their functional diversity (Biswas et al., 2012; Gordon and Taylor, 2005; Wynn et al., 2013). Macrophages undergo profound phenotypic changes when respond to different pathogenic from outside and other microenvironmental cues (Biswas and Mantovani, 2010; Stout and Suttles, 2004), which can be attributed to the clearance of the pathogen.

Unfortunately, the homeostatic and reparative functions of macrophages can lead to tissue with disease states, include cancer, tumour metastasis, metabolic disease and fibrosis (Che et al., 2017; Wynn et al., 2013). Macrophage migration is critical to host immunity (Zhou et al., 2016). It has been demonstrated that macrophages play a critical role in atherosclerosis. Monocytes and macrophages recruitment in the vessel wall initiates atherosclerosis (Boring et al., 1998; Gosling et al., 1999). Macrophages proliferation, accumulation and foam cells transformation promote atherosclerosis progression (Chinetti-Gbaguidi et al., 2015; He et al., 2017; Moore et al., 2013; Tabas, 2010). *Ira Tabas* proposed macrophage death and defective inflammation resolution in atherosclerosis (Tabas, 2010). In contrast, some studies have shown that macrophage accumulation in adipose tissue from in situ proliferation has protective roles in blood glucose and glucose tolerance (Amano et al., 2014; Murano et al., 2008). Therefore, investigation of the regulatory mechanisms involved in macrophages proliferation, apoptosis and migration has important significance for atherosclerosis and other diseases therapy.

Smad ubiquitylation regulatory factor 1 (Smurf1), a member of the HECT-type E3 ubiquitin ligases Nedd4 family, plays a critical role in a variety of processes, such as bone homeostasis, development, cell cycle regulation and tumorigenesis (Narimatsu et al., 2009; Orvedahl et al., 2011; Wei et al., 2017a; Wei et al., 2017b; Yamashita et al., 2005; Zhu et al., 1999). The mammalian Nedd4 family E3s has nine members:

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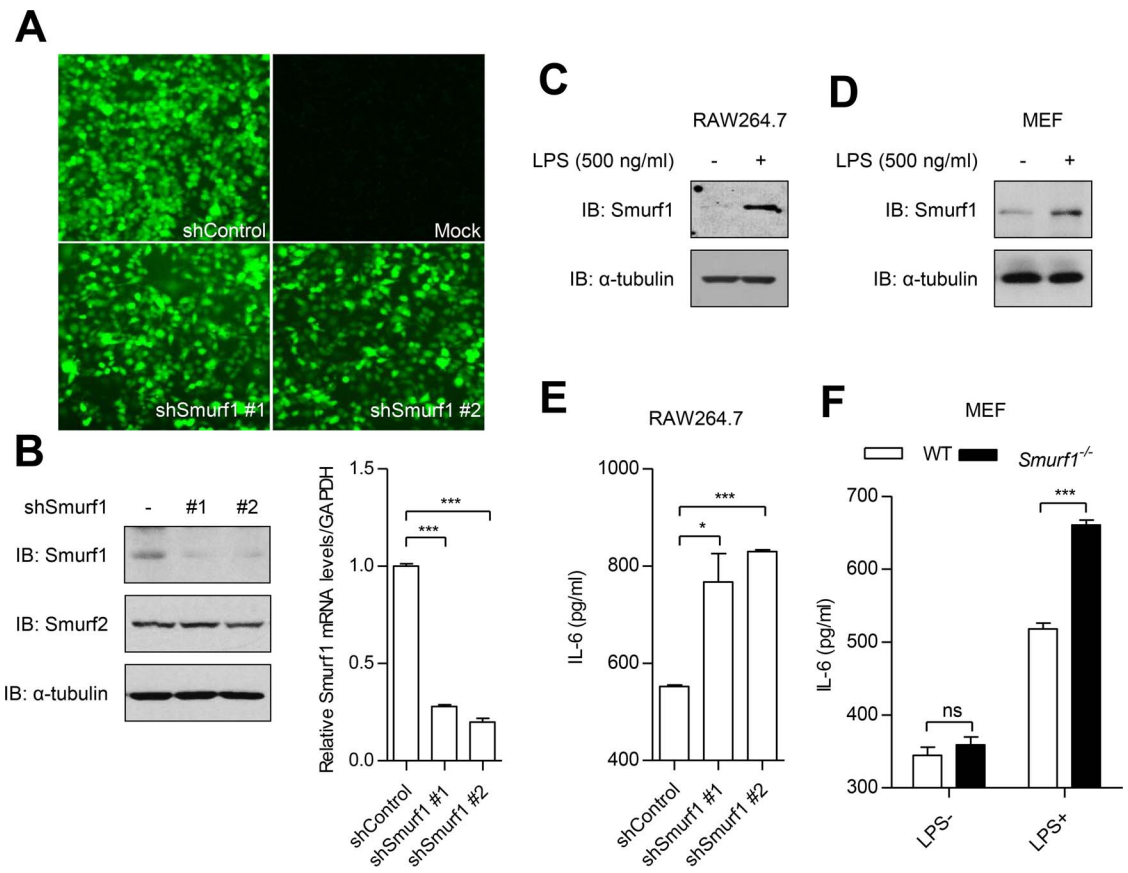

Fig. 1. Smurf1 expression was upregulated in LPS-induced cells.
(A, B) Construction of RAW264.7 cell lines with lentivirus-mediated stably Smurf1 silencing, shSmurf1 #1 and shSmurf1 #2 refer to two knockdown sequences. Green fluorescence micrographs of RAW264.7 cells at 4 days after lentivirus infection for Smurf1 silencing (A). The knockdown efficiency was detected by western blot (B, left) and real-time PCR (B, right). (C, D) Western blot analysis of Smurf1 protein levels in RAW264.7 (C) and MEF (D) cells after treated with 500 ng/ml LPS for 24 h. (E, F) ELISA analysis IL-6 production in shControl, shSmurf1-1 and shSmurf1-2 RAW264.7 cells (E), WT and *Smurf1*$^{-/-}$ MEF cells (F) after treated with 500 ng/ml LPS for 24 h. Representative results from at least three independent experiments are shown. Data are presented as the mean ± s.d. $^{*}P < 0.05$ and $^{***}P < 0.001$, compared to control.

Smurf1, Smurf2, NEDL1, NEDL2, Nedd4-1, Nedd4-2, AIP4/Itch, WWP1 and WWP2 (An et al., 2014; Schwarz et al., 1998). Previously, several studies have demonstrated that Nedd4 family participates in immune regulation. For example, Itch deficiency causes mice develop a systemic and progressive autoimmune disease which results in death at 6 months of age (Matesic et al., 2008). Itch suppresses T cells differentiate into Th2 cells by targeting JunB for ubiquitination-dependent degradation (Fang et al., 2002). The interaction between Itch and Cyld inhibits production of tumor-promoting cytokines by tumor-associated macrophage (Ahmed et al., 2011). Chao Yuan et al. demonstrated that Smurf1 acts as a new negative feedback regulator for IFN-γ signaling through ubiquitination-dependent degradation of STAT1 (Yuan et al., 2012). Casein kinase-2 interacting protein-1 (CKIP-1) interacts with Smurf1 and increases its E3 ligase activity, which plays a critical role in inhibitory regulation of osteogenesis (Lu et al., 2008). We previously demonstrated that CKIP-1 promotes macrophage migration (Zhang et al., 2013). However, the role of Smurf1 in macrophage proliferation, apoptosis and migration remains unknown.

Studies have shown that JNK and p38 MAPK signaling pathways play important regulatory roles in cellular proliferation, apoptosis and migration (Rosa et al., 2016; Senokuchi et al., 2005; Torres and Forman, 2003). Here, we show that Smurf1 expression was elevated in LPS-induced RAW264.7 macrophage and mouse embryonic fibroblasts (MEFs), knockdown of Smurf1 suppresses macrophage proliferation but promotes apoptosis and migration. Furthermore, JNK and p38 MAPK signaling were upregulated in Smurf1-depleted cells. And inhibition of JNK and p38 MAPK signaling in Smurf1 knockdown cells rescue the phenotypes of macrophage proliferation, apoptosis and migration. Therefore, our results demonstrate that Smurf1 is a new positive regulator for macrophage proliferation and apoptosis, but a negative regulator for macrophage migration.

2. Materials and methods

2.1. Cell culture and cell lines

RAW264.7 cells and BMDMs were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA), while MEF cells were grown in DMEM (Gibco, Grand Island, NY, USA), both plus with 10% fetal bovine serum (FBS), 100 units ml^{-1} penicillin and streptomycin, at 37 °C in a humidified 5% CO_2 incubator.

To knock down endogenous Smurf1 expression, lentiviruses containing Smurf1 or control shRNA were purchased from GeneChem Co., Ltd., Shanghai, China. And the stable cell lines with Smurf1 knockdown were constructed. In brief, 1×10^6 RAW264.7 cells were cultured in six-well plates for 18 h before lentiviruses infection. After 12 h, cells were cultured in fresh medium for 3–4 days. And then 4 μg/ml puromycin was used to screen the positive cells, transfected with lentiviruses and expressed green fluorescent protein (GFP), for 2–3 days. The knockdown efficiency was detected by western blot and real-time PCR.

2.2. Western blot analysis

RAW264.7 or MEF cells were lysed in RIPA lysis buffer (Sigma, Darmstadt, Germany) supplemented with protease inhibitor cocktail