

Gelatin promotes cell aggregation and pro-inflammatory cytokine production in PMA-stimulated U937 cells by augmenting endocytosis-autophagy pathway

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ABSTRACT

Gelatin, denatured collagen, temporarily exists in tissues and may well be pathophysiologically involved in tissue remodeling, inflammation or tissue damage. The present study is aimed to investigate possible biological roles of gelatin by examining its effects on monocyte-like histiocytic lymphoma cell line U937. Once stimulated by phorbol 12-myristate 13-acetate (PMA), U937 cells differentiate into macrophage-like cells, changing from non-adherent to adherent cells with extended pseudopodia. Here we pre-treated the cell dishes with gelatin solution for cell culture. Interestingly, we found that PMA-stimulated U937 cells formed multicellular aggregates on gelatin-coated dishes, accompanying NF-κB-mediated production of pro-inflammatory cytokines, whereas cell aggregation was not detected on non-coated dishes. Moreover, differentiated U937 cells on gelatin-coated dishes showed increased autophagy level and endocytosis. Surprisingly, formation of multicellular aggregates and pro-inflammatory cytokine production were both attenuated by either down-regulation of autophagy with inhibitors, such as 3-methyladenine (3MA) or chloroquine (CQ), or repression of endocytosis with siRNA targeting Endo180. Moreover, autophagy was inhibited by si-Endo180, and endocytosis was suppressed by 3MA, suggesting a positive feedback loop between autophagy and endocytosis. The results revealed that gelatin-coating induced differentiated U937 cells to form cell aggregates and promote NF-κB-mediated pro-inflammatory cytokine production at least partially through an endocytosis-autophagy pathway.

1. Introduction

Extracellular matrix (ECM) is a dynamic complex consisting of macromolecules including proteoglycans, glycoproteins, collagens, growth factors, cytokines and proteases (Kanta, 2015; Olczyk et al., 2014), serving as the main component of microenvironment that supports tissue architecture (Adair-Kirk and Senior, 2008; Valiente-Alandi et al., 2016). In addition, components of ECM are known to affect cell adhesion, shape, migration, proliferation, survival and differentiation (Byron et al., 2013).

Collagens are the main structural components of ECM. Type I collagen is the most abundant protein in human body (Han et al., 2015; Ricard-Blum, 2011). Collagen as other macromolecules undergoes a dynamic process with ongoing synthesis and degradation at any time point (McGavigan et al., 2006). *In vivo*, matrix metalloproteinases (MMPs) primarily participate in collagen degradation (Hijmans et al., 2017; Rosenblum et al., 2010). Degradation of collagens significantly increases in various pathological situations such as tissue remodeling, inflammation and other tissue impairments (Zeng et al., 2011). Up-regulation of MMPs expression results in collagen breakdown in

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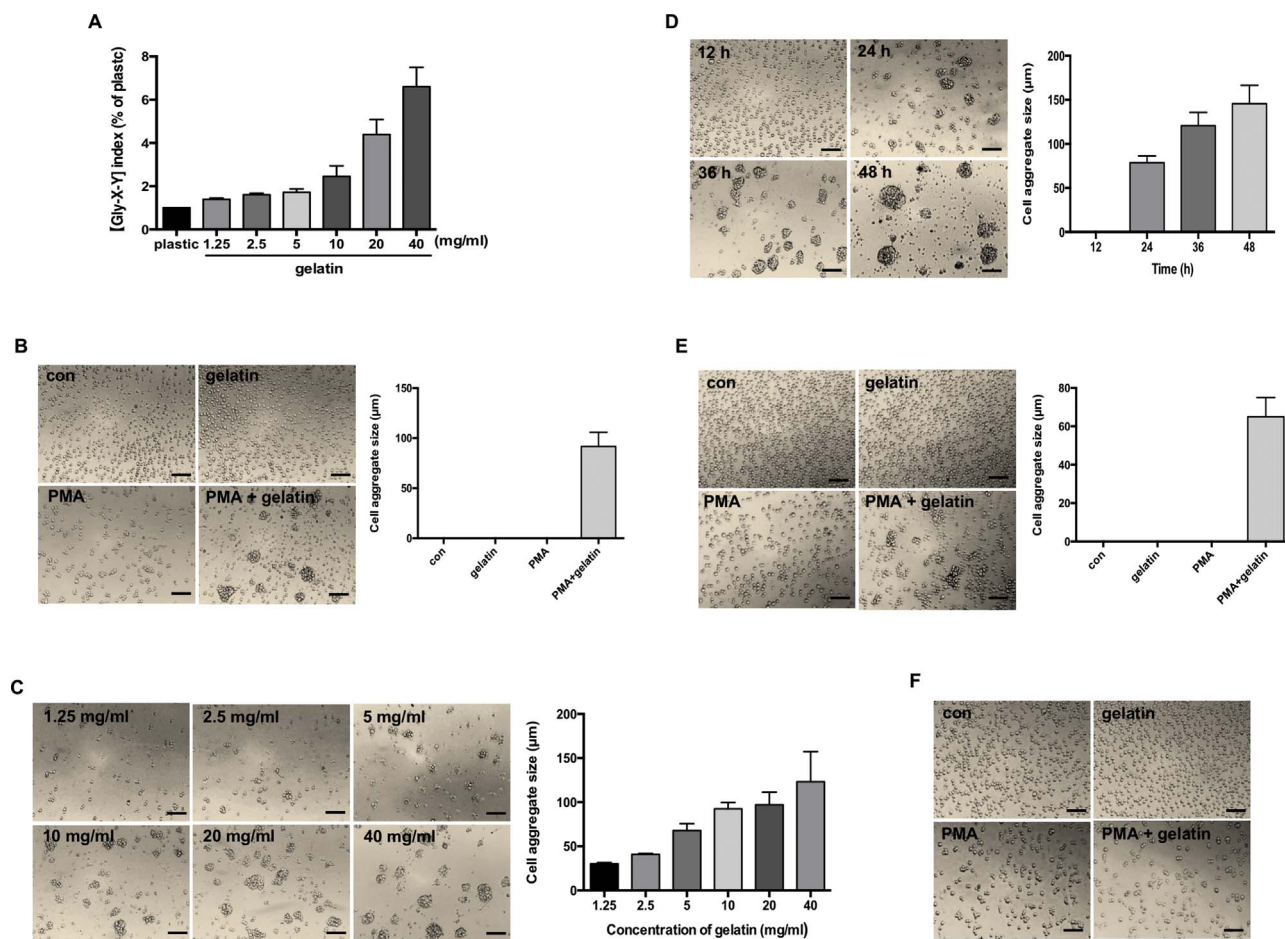


Fig. 1. PMA-treated U937 cells formed multicellular aggregates on gelatin-coated culture. (A) Relative gelatin amount coated as expressed by fold of plastic. (B) to (F) Morphological images by phase contrast microscopy (Scale bar = 100 μm). Gelatin concentration was 10 mg/ml. (B) to (E) Size of cell aggregates. Data are means from three random fields under microscope images. (B) Effect of PMA treatment and collagen-coating on U937 cells. (C) Effect of gelatin concentration (Scale bar = 100 μm). Data are means from three random fields under microscopic images. (D) Time dependency. Images. (E) The effect of gelatin added to cell culture at 12 h without precoating with gelatin. Morphological images. (F) The effect of gelatin added to cell culture at 24 h without precoating with gelatin.

myocardial infarction (Villarreal et al., 2004). Furthermore, polypeptide fragments are released from denatured collagen in skin burn (Stevens et al., 2002b; Yu et al., 2016). Gelatin is derived from denaturation of triple-helical collagen or its fragments (Menon et al., 2013; Yu et al., 2016).

Since gelatin is produced along with inflammation, we hypothesize that gelatin might affect the progression of inflammation, as ECM fragments affect inflammation by releasing cytokines (Dai et al., 2010; O'Reilly et al., 2008; Yu et al., 2016). It is reported that collagen fragments augment or suppress IL-1β production, depending on different culture conditions (Adair-Kirk and Senior, 2008; O'Reilly et al., 2008). In addition, denatured collagen mimicks IL-8 in the effect through their structural homology (Weathington et al., 2006). However, there are only a few studies on gelatin-associated inflammatory responses (Brigo et al., 2017; Horii et al., 2017; Wu et al., 2017); therefore, the effect of gelatin on differentiated human acute myelogenous leukemia U937 cells was focused in this study. We found that differentiated U937 cells formed cell aggregates on gelatin-coated dishes, releasing pro-inflammatory cytokines via NF-κB pathway. We describe the evidence that gelatin-induced changes of PMA-activated U937 cells are attributed at least partially to gelatin-augmented endocytosis-autophagy pathway.

2. Materials and methods

2.1. Reagents

Monodansylcadaverine (MDC), 3-methyladenine (3MA), chloroquine (CQ), phorbol 12-myristate 13-acetate (PMA), pyrrolidine dithiocarbamate (PDTC), and rabbit monoclonal anti-LC3 antibody were obtained from Sigma Chemical (St. Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) diagnostic kits of human IL-1β, TNFα and PGE2 were purchased from Dakewe Biotech (Shenzhen, Guangdong, China). Human Beclin 1-targeted (si-Beclin 1), Endo180-targeted (si-Endo180) and negative control (si-con) siRNA were purchased from GenePharma (Suzhou, Jiangsu, China). Primary antibodies against α-tubulin, IL-1β, TNFα, cyclooxygenase-2 (COX-2), Beclin 1, Atg5 and Endo180 as well as horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Electrochemiluminescence (ECL) reagent was from Thermo Scientific (Rockford, IL, USA).

2.2. Cell culture on gelatin-coated plates

Gelatin is from Nippi Research Institute of Biomatrix (Toride, Ibaraki, Japan). The gelatin was extracted from porcine skin by hot water at 50–60 °C, and was purified by filtration and ion-exchange chromatography. After purification, gelatin solution was sterilized with filtration. Prior to cell culture, gelatin solution diluted with 0.5 mM

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