Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Cell Research 311 (2005) 39 – 48

Experimental Cell Research

www.elsevier.com/locate/yexcr

Research Article

TNF-α promotes cell survival through stimulation of K^+ channel and NFκB activity in corneal epithelial cells

Ling Wang[a], Peter Reinach[b], Luo Lu[a,*]

[a]*Division of Molecular Medicine, Harbor-UCLA Medical Center, David Geffen School of Medicine, University of California Los Angeles, 1124 W. Carson Street, C-2, Torrance, CA 90502, USA*

[b]*Department of Biological Sciences, SUNY College of Optometry, New York, NY 10010, USA*

Received 8 April 2005, revised version received 18 August 2005, accepted 18 August 2005
Available online 10 October 2005

Abstract

Tumor necrosis factor (TNF-α) in various cell types induces either cell death or mitogenesis through different signaling pathways. In the present study, we determined in human corneal epithelial cells how TNF-α also promotes cell survival. Human corneal epithelial (HCE) cells were cultured in DMEM/F-12 medium containing 10% FBS. TNF-α stimulation induced activation of a voltage-gated K^+ channel detected by measuring single channel activity using patch clamp techniques. The effect of TNF-α on downstream events included NFκB nuclear translocation and increases in DNA binding activities, but did not elicit ERK, JNK, or p38 limb signaling activation. TNF-α induced increases in p21 expression resulting in partial cell cycle attenuation in the G_1 phase. Cell cycle progression was also mapped by flow cytometer analysis. Blockade of TNF-α-induced K^+ channel activity effectively prevented NFκB nuclear translocation and binding to DNA, diminishing the cell-survival protective effect of TNF-α. In conclusion, TNF-α promotes survival of HCE cells through sequential stimulation of K^+ channel and NFκB activities. This response to TNF-α is dependent on stimulating K^+ channel activity because following suppression of K^+ channel activity TNF-α failed to activate NFκB nuclear translocation and binding to nuclear DNA.

Keywords: Patch clamp; K^+ channel blockers; Cell death; Cell cycle mapping

Introduction

TNF-α has pleiotropic effects in many cell types that include activation of apoptosis, inflammation, and immune responses. These responses underlie the pathogenesis of various diseases, including diabetes and cancer. Conversely, TNF-α can also induce through different signaling pathway module proliferation and differentiation [1]. TNF-α is one of a number of cytokines found in tears and its expression is elevated in allergic conjunctivitis [2]. TNF-α is also secreted by corneal epithelial cells in response to inflammatory stimulation caused by bacterial and viral infections [3–6]. However, a cell survival-promoting role for TNF-α has not been described in this tissue.

The TNF-α-linked cell signaling pathways that elicit apoptosis have been extensively characterized. Upon binding of TNF-α to Tumor Necrosis Factor Receptor 1 (TNF-R1), a type I membrane protein in the TNFR superfamily in mammalian cells, TNF-R1 monomers cluster resulting in death signal initiation [7–10]. There is a common structure among these receptors known as the "death domain" (DD) on the cytoplasmic side of the membrane [10]. Activation of a DD-containing receptor by TNF-α can lead to recruitment of intracellular DD-containing adaptors such as Fas associated death domain (FADD) and TNFR associated death domain (TRADD). TNF-α activates TNF-R1 coupled receptor DDs and they recruit several signaling molecules (adaptor proteins) to the activated receptor including: FADD, TRAFs, and receptor-interaction protein (RIP) leading to activation of caspase 8, NFκB, and c-Jun N-terminal kinase (JNK) signaling pathways [11,12]. NFκB protein is a member of the cytoplasmic heterodimeric

* Corresponding author. Fax: +1 310 222 3781.
E-mail address: lluou@ucla.edu (L. Lu).

0014-4827/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2005.08.020

transcription factor family associated with cell survival [13,14]. Upon phosphorylation by IKK, NFκB dissociates from IκB and IκB is then degraded by proteosome complexes [15]. Unbound NFκBs dimerize and become active transcription factors. They then translocate into the nucleus to initiate gene transcription in response to TNF-α.

Even though TNF-α induces proliferation in some cell types, a cell signaling role for TNF-α-induced K^+ channel activation in eliciting this response has not been described. Their involvement is tenable because K^+ channels are a component of signaling pathways that mediate mitogenic responses to serum-containing growth factors in many cell types including corneal epithelial cells [16–19]. K^+ channel activity belonging to the Kv3.4 family in these cells is essential for EGF-induced proliferation [16,20]. Inhibition of K^+ channel activity with specific channel blockers results in attenuation of the cell cycle in the G_1 phase [16–19]. On the other hand, UV irradiation also induces robust increases in channel activity in these cells, which in turn triggers apoptosis through activation of JNK signal cascades [20–22]. TNF-α-stimulated increases in K^+ channel activities are important for TNF-α-induced cellular effects in cortical neurons, kidney epithelial, and liver cells [23–25]. TNF-α also induces mRNA expression of various K^+ channel types during a systemic inflammatory response as well as tumor cell proliferation in brain and cancer cells [26–28]. However, TNF-α suppresses particular types of K^+ channel activity in other cell types, indicating that the effect of TNF-α on K^+ channel activity is dependent on the channel type and cell origins [26,29]. In the present study, we investigated the role of TNF-α-induced changes in K^+ channel activity in determining human corneal epithelial cell fate. Our results reveal that such changes are requisite for TNF-α-induced NFκB nuclear translocation and activation, which in turn improves corneal epithelial cell survival. On the other hand, TNF-α-induced cellular responses in these cells do not involve activation of any of the MAP kinase limb pathways. It is currently unclear why these MAP kinase pathways are not involved in mediating improved cell survival in response to TNF-α exposure.

Methods

Cell culture of corneal epithelial cells

SV40-immortalized human corneal epithelial (HCE) cells were grown in DMEM/F-12 culture medium containing 10% fetal bovine serum, 5 μg/ml insulin, and 10,000 units/ml penicillin and 10,000 μg/ml streptomycin, and maintained in an incubator supplied with 95% air and 5% CO_2 at 37°C. Before experimentation, cells were synchronized in the G_1 phase of the cell cycle by serum deprivation for at least 24 h. The medium was replaced every 2 days and the cells were passed by treatment with 0.05% trypsin-EDTA. For apoptosis induction, cells were placed 6 cm away from a safe light containing UV-C in a tissue culture hood and irradiated with 40 μJ/cm^2. After irradiation, they were collected and rinsed with phosphate-buffered saline (PBS). To suppress K^+ channel activity, the cells were exposed to a K^+ channel blocker, 4-aminopyridine (4-AP) and preincubated for 20 min prior to exposure to TNF-α dependent on the time requirement of different experiments. All chemicals unless otherwise specified were obtained from Sigma Chemicals.

Nuclear staining with ethidium bromide and acridine orange

Cell viability and apoptosis were determined by nuclear staining to detect nuclear DNA condensation. A dye mixture containing ethidium bromide and acridine orange, each of which was present at 100 μg/ml (EB/AO), was added to each cell culture dish at a final density of 3×10^5/ml. Cell populations were scored according to their staining color and intensity using a UV-fluorescence microscope (Nikon). Nuclei staining green have not lost membrane integrity. In contrast, corneal epithelial cells in which the nuclei stained orange have lost membrane integrity. Apoptotic cells can be distinguished from viable cells on the basis of the absence or presence of nuclear condensation/fragmentation.

Immunoblot analysis

The cells (2×10^5) were rinsed twice with ice-cold PBS and solubilized in sodium dodecyl sulfate polyacrylamide (SDS) sample buffer containing 62.5 mM Tris–HCl pH 6.8, 2% W/V SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue, or phenol red. The resulting suspensions were denatured by boiling for 5 min. After fractionation of cell lysates with 12% polyacrylamide gel (PAGE), proteins were electrotransferred to PVDF membranes. Membranes were exposed to blocking buffer containing 5% nonfat milk in TBS-0.1% Tween 20 (TBS-T) for 1 h at room temperature (RT, 21–23°C), and then incubated overnight or for 1 h with antibodies of interest at 4°C. All antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). After three washes with TBS-T buffer, membranes were incubated with alkaline phosphatase (AP)-linked secondary antibody for 1 h at RT. The proteins were detected with a Phototope-Star Western Blot Detection kit (Cell Signaling Technology, Beverly, MA). In addition, caspase 3 activity was detected to determine cell apoptosis by using an antibody against cleaved caspase 3. This antibody recognizes endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175 (Cell Signaling Technology, Beverly, MA).

Electrophoretic mobility shift assay (EMSA)

Nuclear proteins were extracted by centrifugation at 25 K rpm for 20 min. The consensus oligonucleotide for NFκB transcription factor (5′-AGTTGAGGGGACTTTCCCAG-