Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

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

Short communication

Proinflammatory cytokine interferon-γ increases the expression of BANCR, a long non-coding RNA, in retinal pigment epithelial cells

R. Krishnan Kutty[a,*], William Samuel[a], Todd Duncan[a], Olga Postnikova[a], Cynthia Jaworski[a], Chandrasekharam N. Nagineni[b], T. Michael Redmond[a]

[a] Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892, United States
[b] Radiation Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States

ARTICLE INFO

Keywords:
BRAF-activated non-coding RNA (BANCR)
Long non-coding RNA (lncRNA)
Interferon-γ
JAK-STAT1 signaling
Retinal pigment epithelium
Age-related macular degeneration

ABSTRACT

The inflammatory response may contribute to retinal pigment epithelial (RPE) dysfunction associated with the pathogenesis of age-related macular degeneration (AMD). We investigated whether the inflammatory response affects the expression of long coding RNAs (lncRNAs) in human RPE-derived ARPE-19 cells. This class of regulatory RNA molecules recently came to prominence due to their involvement in many pathophysiological processes. A proinflammatory cytokine mixture consisting of IFN-γ, IL-1β and TNF-α altered the expression several lncRNAs including BANCR in these cells. The cytokine responsible for increasing BANCR expression in ARPE-19 cells was found to be IFN-γ. BANCR expression induced by IFN-γ was suppressed when STAT1 phosphorylation was blocked by JAK inhibitor 1. Thus, proinflammatory cytokines could modulate the expression of lncRNAs in RPE cells and IFN-γ could upregulate the expression of BANCR by activating JAK-STAT1 signaling pathway.

1. Introduction

Recent studies have shown that long non-coding RNAs (lncRNAs), non-protein coding transcripts with a minimum length of 200 nucleotides, may mediate many pathophysiological processes including inflammatory response [1,2]. BRAF-activated non-coding RNA (BANCR, LINC00586), a 693-nucleotide long lncRNA transcribed from human chromosome 9, has been shown to regulate proliferation and migration of melanoma and other cancer cells [3–5]. This lncRNA is also reported to regulate epithelial-mesenchymal transition (EMT) [4,5]. Furthermore, BANCR is highly expressed in retinoblastoma tissues and cells, and silencing its expression resulted in a decrease in retinoblastoma cell proliferation, migration and invasion [6].

Retinal pigment epithelium (RPE), a polarized monolayer of pigmented cells located adjacent to photoreceptor cells, is indispensable for visual function since it provides nutrients to the photoreceptor cells, regenerates the visual chromophore 11-*cis*-retinal and phagocytoses rod outer segment discs generated by circadian shedding [7]. Dysfunction of RPE resulting from abnormal inflammatory response may play a role in the pathology of age-related macular degeneration (AMD) [8,9]. Proinflammatory cytokines secreted by infiltrating macrophages and lymphocytes may trigger retinal pigment epithelial (RPE) cell dysfunction. We have reported earlier that human RPE derived ARPE-19 cells respond to proinflammatory cytokines IFN-γ, TNF-α and IL-1β by increasing the expression of cytokines and chemokines [10]. The proinflammatory cytokines also decreased the expression of RPE characteristic genes and induced epithelial-mesenchymal transition (EMT)-like changes in these cells [11]. It is not yet known whether lncRNAs play a role in mediating the deleterious effect of IFN-γ, TNF-α and IL-1β on RPE cells. Therefore, we investigated whether these proinflammatory cytokines can affect the expression of lncRNAs like BANCR in the ARPE-19 cells. We observed that the inflammatory response altered the expression of several lncRNAs in these cells and that the activation of JAK-STAT1 signaling pathway by IFN-γ increased the expression of BANCR.

2. Materials and methods

2.1. Cell culture

Human ARPE-19 cells were cultured until they exhibited RPE characteristics as recently described from our laboratory [11–13]. Briefly, the cells were grown in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, 4 mM L-glutamine, 1 mM sodium pyruvate,

* Corresponding author at: Laboratory of Retinal Cell and Molecular Biology, Bldg. 6, Room 112, National Eye Institute, National Institutes of Health, 6 Center Dr., MSC 0608, Bethesda, MD 20892, United States.
E-mail address: kuttyk@nei.nih.gov (R.K. Kutty).

http://dx.doi.org/10.1016/j.cyto.2017.10.009
Received 15 June 2017; Received in revised form 5 October 2017; Accepted 8 October 2017
1043-4666/ Published by Elsevier Ltd.

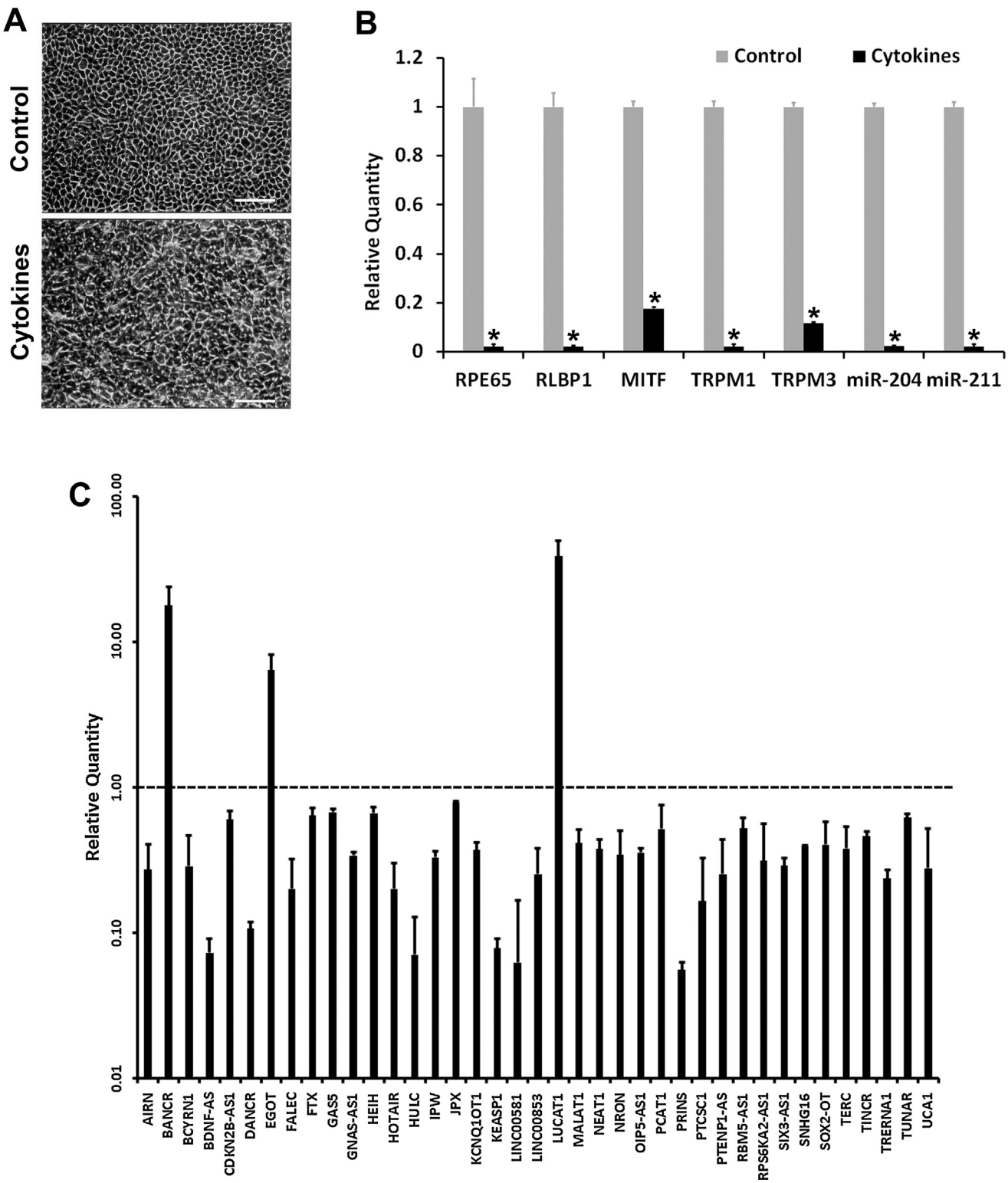

Fig. 1. Proinflammatory cytokines alter the expression of lncRNAs in RPE cells. Differentiated ARPE-19 cells were treated for 4 days with a proinflammatory cytokine mixture consisting of IFN-γ (100 u/ml), TNF-α (10 ng/ml) and IL-1β (10 ng/ml). (A), Phase contrast microscopic images of control and treated cells shows that proinflammatory cytokines caused a disruption in the epithelial morphology of ARPE-19 cells. Photomicrographs were taken at a magnification of 100×; scale bar = 100 μm. (B) Real-time PCR analysis shows that proinflammatory cytokines decrease the expression of RPE characteristic genes and miRNAs in ARPE-19 cells. * = $p < .05$ when compared to control, n = 3. (C) Real-time PCR analysis shows that proinflammatory cytokines alter the expression of lncRNAs in ARPE-19 cells. Relative expression level of lncRNAs affected by the treatment are shown ($p < .05$ when compared to control; n = 3). Broken line indicates that the relative expression level of an lncRNA in the control (untreated cells) is 1.

100 U/ml penicillin, 100 μg/ml streptomycin and 1% fetal bovine serum at 37 °C in a humidified environment of 5% CO_2. The culture medium was replaced twice every week. The cells kept under these conditions for 4 months exhibited RPE characteristics such as pigmentation, epithelial morphology and expressed visual cycle genes. The cells were treated with the proinflammatory cytokine mixture consisting of IFN-γ (100 u/ml), IL-1β (10 ng/ml) and TNF-α (10 ng/ml) in the presence of serum for 4 days unless indicated otherwise. Human