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Involvement of mitogen-activated protein kinases and protein kinase C in regulation of antioxidant response element activity in human keratinocytes

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Abstract

Antioxidant response element (ARE) is a unique *cis*-acting regulatory sequence located in the upstream regions of many genes encoding anticarcinogenic/antioxidant proteins. Induction of ARE dependent genes plays an important role in protection of cells against oxidative damage. However, the signaling mechanism(s) involved in regulating transcription of ARE dependent gene expression has not been clearly defined. In this study, we identified protein kinases that are involved in regulation of ARE activity by using specific pharmacological inhibitors of protein kinases in engineered human HaCaT keratinocytes, which stably express the ARE-driven green fluorescent protein (GFP) as a reporter. When HaCaT/GFP cells were treated with *tert*-butylhydroquinone (tBHQ), a well-known ARE activator, GFP expression was up-regulated in time and dose dependent manner, indicating that tBHQ activates the ARE in these cells. Treatment of cells with SB202190 (a specific inhibitor of p38), staurosporine (a wide-spectrum inhibitor of PKC) or rottlerin (a specific inhibitor of PKCδ) all augmented ARE activation by tBHQ. These results suggest that p38 and PKC, especially PKCδ, play inhibitory roles in ARE activation in human keratinocytes. Furthermore, UVB irradiation minimally affects the basal ARE activity but significantly suppresses tBHQ induced ARE activation, indicating that UVB irradiation interrupts tBHQ signaling. Interestingly, treatment of HaCaT/GFP cells with SP600125 (a specific inhibitor of JNK) could reverse UVB mediated suppression of ARE activation by tBHQ. This suggests that the suppressive effect of UVB on ARE activation by tBHQ is mediated by a JNK pathway(s). These findings provide useful information for developing novel strategies for skin cancer chemoprotection through ARE activation.
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Keywords: Antioxidant response element; HaCaT; Signal transduction; Protein kinases; UVB

1. Introduction

Ultraviolet (UV) exposure, particularly UVB, is a major etiological factor for the vast majority of cutaneous malignancies diagnosed in the human population [1]. In addition to directly causing DNA damage, UVB generates reactive oxygen species (ROS) that can lead to oxidative stress, when their formation exceeds the antioxidant defense ability of the target

Abbreviations ARE, antioxidant response element; GFP, green fluorescent protein; ROS, reactive oxygen species; UVB, ultraviolet B.

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cells [2,3]. Studies have shown that ROS can act as both tumor initiator and promoter by damaging critical cellular macromolecules and by acting as an inducer of cell-signaling molecules [4].

It is known that UV exposure inactivates or depletes cellular antioxidants including enzymatic and non-enzymatic antioxidants in the epidermis and in cultured keratinocytes. Cellular antioxidants play a significant role in ameliorating or preventing photo-biological damage [5,6]. Induction of antioxidant response element (ARE) dependent protective genes has been considered as a promising strategy for cancer chemo-prevention [7,8], because products of these genes, including phase II detoxification enzymes and other antioxidants proteins, could provide cellular protection against oxidative damage by directly scavenging ROS, detoxifying harmful products formed in the oxidative reaction cascades as well as exporting the detoxification products out of cells. Importantly, these genes can be up-regulated by many naturally occurring or synthetic chemical compounds, such as sulforaphane and *tert*-butylhydroquinone (tBHQ), which have been shown to possess both anticarcinogenic and neuroprotective properties [9,10].

The antioxidant response element (ARE) is a unique *cis*-acting regulatory sequence, defined as 5′-TGACnnnGC-3′ and identified in the promoter regions of several genes encoding phase II detoxification enzymes, and antioxidant proteins such as NAD(P)H:quinone oxidoreductase-1 (NQO1), glutathione *S*-transferases (GSTs), glutamate–cysteine ligase (GCL), heme oxygenase-1, ferritin, and thioredoxin [11]. The mechanism by which oxidative and electrophile stress evokes an ARE response has been investigated extensively. Studies demonstrate that transcriptional regulation of these genes is mediated via an ARE. It has been shown that transcription factor erythroid 2 p45-related factor 2 (Nrf2) is essential for ARE-mediated induction [12]. Under normal conditions, Nrf2 is sequestered in the cytoplasm by actin binding protein Keap-1. Upon treatment of cells with stimuli, Nrf2 dissociates from Keap-1 and translocates to nucleus, where Nrf2 heterodimerizes with small Maf proteins and binds to the ARE, and activates transcription of ARE dependent genes [13]. However, how a cell senses and responds to appropriate signals to initiate the cascade of signaling events leading to the activation or suppression of the ARE is not clearly understood. To date, three major signal transduction pathways have been implicated in the regulation of the ARE activity. They are mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) pathways [14–16]. However, involvement of these protein kinases in the regulation of ARE activity appears to vary with cell types. We have, therefore, decided to examine the roles of these kinases in human skin keratinocytes.

MAPKs belong to a large family of serine/threonine protein kinases comprising three distinct families: extracellular-signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAP kinases. The MAPKs are part of essential signal transduction cascades that regulate cell growth, differentiation, apoptosis and transformation [17]. PI3K is a lipid kinase that phosphorylates phosphatidylinositols at the 3-position of the inositol ring. This enzyme has been found to be associated with activation of cellular survival signals in response to several growth factors and has been implicated in mitogenesis and cell transformation [18]. PKC is a large family of serine/threonine protein kinases classified into three subfamilies: conventional PKC (cPKC) which are calcium-responsive and include cPKCα, -βI, -βII, and -γ; novel PKC (nPKC), which do not respond to calcium and include nPKCδ, -ε, and -η; and atypical PKC (aPKC), which are not affected by calcium, phorbol esters, or diacylglycerol and include aPKCζ and -ι. Studies have demonstrated that PKC isotypes play distinct roles in the regulation of growth and differentiation, depending on which cell types are being examined [19,20].

MAPKs, PI3K, as well as PKC isotypes such as α, βI, βII, δ, ε, ζ, and η have been found to be expressed in human keratinocytes [21–23]. Because of the conflicting reports concerning the involvement of protein kinases in the induction of ARE dependent genes, and since no information is available regarding the effect of UVB irradiation-induced activation of protein kinases on transcriptional regulation of ARE-dependent genes, we carried out experiments to identify the signaling molecules involved in either tBHQ mediated up-regulation or UVB-mediated down-regulation of ARE activity. We established a human HaCaT keratinocyte cell line which stably expresses the ARE driven green fluorescent protein (GFP) as a reporter of ARE activity. Application of this HaCaT/GFP cell line and pharmacological inhibitors allowed us to efficiently identify the protein kinases that are involved in the regulation of ARE activity. Our results showed that inhibition of p38 kinase and PKC augments the tBHQ induced ARE activation in HaCaT cells and inhibition of JNK abolishes the inhibitory effect of UVB on ARE activation by tBHQ.

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