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Protective effect of oxidative stress in HaCaT keratinocytes expressing E7 oncogene

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Summary. In a previous study, we established a stable cell line which constitutively expresses E7 in HaCaT human keratinocyte cell line and identified various relevant factors including oxygen modulators affected by the E7 oncogene. E7-expressing HaCaT cells (HaCaT/E7) appeared to be more resistant to H_2O_2-induced cell death. Here, we demonstrate how E7 oncogene would modulate oxidative stress-induced cell death. In addition, we verified the increased expression of catalase in the HaCaT/E7 by Western blot analysis. The results suggest that the E7 oncogene would induce higher resistance to ROS-induced cell injury in the E7-infected cells via the upregulation of catalase. To investigate these paradoxical effects of high concentrations of H_2O_2 (500 μM–1 mM), we examined their effects on receptor mediated apoptosis, cell death via the mitochondrial pathway and modulation of apoptosis related factors. Our results revealed that HaCaT keratinocytes infected with HPV 16 E7 oncogene modulated expressions of catalase, Bcl-xL, IL-18, Fas, Bad, and cytochrome c as well as NF-κB, resulting in the resistance to oxidative stress-induced cell death.

Keywords: Human papillomavirus – E7 – Apoptosis signal

Abbreviations: EMSA, electrophoretic mobility shift assay; HaCaT/E7, HaCaT keratinocytes expressing E7 oncogene; HPV, human papillomavirus; pRb, retinoblastoma; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction

Introduction

Human papillomaviruses (HPVs) are small double-stranded DNA viruses, which induce hyperproliferative lesions of cutaneous and mucosal epithelia. E7 alone can cooperate not only to immortalize human keratinocytes (Halbert et al., 1991) but also to promote the formation of benign tumors (Song et al., 2000). In contrast, E7 is only weakly capable of inducing gross chromosomal alterations, such as aneuploidy, that are indicative of malignancy (Reznikoff et al., 1994). Cells contain antioxidant enzymes to maintain steady levels of ROS produced by metabolic products (Cullen et al., 2003). Among the antioxidants, catalase is a heme-containing peroxisomal enzyme that breaks down hydrogen peroxide into water and oxygen; and protects hemoglobin by removing H_2O_2 generated in normal human erythrocytes, which are exposed to substantial oxygen concentrations (Gaetani et al., 1989). The net intracellular concentration of ROS is determined by the balance between its production and the ability of antioxidants to remove them.

The redox regulator catalase is required for the redox potential of the cell and exerts important functions in cell apoptosis. Increased catalase activity has been shown to prevent cell death via the receptor mediated apoptotic pathway as well as cell death via the mitochondrial pathway. The present state of the literature reveals that antioxidant enzymes play a more intricate role in cell physiology than previously assumed (Kahl et al., 2004).

H_2O_2 induces different cellular effects depending on concentration and cell type. Several studies have been documented regarding the involvement of oxidative stress in the process of apoptosis (Parman et al., 1999). H_2O_2 has been shown to act as a signaling molecule involved in

many cellular functions such as apoptosis and proliferation. Apoptosis is recognized as a chemotheapeutic cell death. It is usually induced via cell surface receptors such as TNF receptor family and Fas (APO-1, CD95) by stresses. Actually, Fas plays a role in examples of chemotherapy-induced apoptosis in various cell types (Kaufmann, 1989; Kaufmann et al., 1993).

Cell death plays an important role in regulating tissue homeostasis. Programmed cell death takes place during the process of development and is essential for the orderly formation of tissues or organs. During post-natal development, cell death often occurs to eliminate damaged cells. Necrosis and apoptosis are cell death observed after injury or toxic stress. These two-different types of cell death can be discriminated by morphological and biochemical criteria (Majno and Joris, 1995; Thompson, 1995; Hale et al., 1996). A release of cytochrome c from mitochondria causes an activation of caspase 9, which initiates a cascade of proteolytic cleavage carried out by multiple caspases (Green and Reed, 1998), that is, activation of caspases as a result of cytochrome c release can lead to degradation of cellular components and finally cell death. Bax and bad protein can cause the formation of mitochondrial membrane channels that release cytochrome c, whereas bcl-2 protein has been shown to prevent mitochondrial release of cytochrome c (Adams and Cory, 1998; Chao and Korsmeyer, 1998).

In the previous study (Shim et al., 2005), we established a stable cell line expressing E7, using the human keratinocyte HaCaT cell line. The increased expression of catalase, one of the typical anti-oxidant enzymes, was verified via matrix-assisted laser desorption/ionization time of flight and Western blot analysis in the E7-expressing HaCaT cells (HaCaT/E7). HaCaT/E7 cells appeared to be more resistant to H_2O_2-induced cell death. In the present study, we focused on the mechanism by which E7 oncogene would modulate oxidative stress-induced cell death in HaCaT keratinocytes infected with HPV 16 E7 oncogene.

Materials and methods

Materials

Dulbecco's Modified Eagle Medium (DMEM) was purchased from GIBCO/BRL (Rockville, MD, USA) and supplemented with 10 mM L-glutamine, 24 mM $NaHCO_3$, 10 mM Hepes, 100 units/ml penicillin, and 100 μg/ml streptomycin (GIBCO/BRL). The following antibodies were used: anti-catalase (Calbiochem, San Diego, CA, USA), anti-IκBα (Santa Cruz Biotechnology), anti-α-tubulin (Sigma, Steinherm, Germany), anti-Fas ligand (FasL) (Transduction Laboratories, Lexington, KY, USA), anti-Fas (MBL, Nagoya, Japan), anti-Bad (Santa Cruz Biotechnology), anti-Bcl-xL (Santa Cruz Biotechnology), anti-cytochrome c (BD Biosciences, Oxford, UK), horseradish peroxidase conjugated anti-goat IgG (Santa Cruz Biotechnology), horseradish peroxidase conjugated anti-mouse IgG (Sigma), and alkaline phosphatase conjugate anti-rabbit IgG (Sigma).

Cell culture and establishment of stable E7 transfectants

Human keratinocyte cell line HaCaT was maintained in DMEM containing 10% heat-inactivated fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) as described (Boukamp et al., 1988). HaCaT was used to establish mock control- and E7-expressing stable cell lines. In brief, the pOPRSVICAT vector and pOPRSVICAT vector containing E7 were transfected into HaCaT cells and stable transfectants were selected by allowing to grow under selective DMEM containing G-418 (0.8 mg/ml) for months, as previously described (Cho et al., 2001).

Sample preparation

Cells were lysed in a lysis buffer (25 mM Tris–phosphate, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid, 10% glycerol, 1% Triton X-100, pH 7.8) containing aprotinin (10 μg/ml) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). The total cell lysates were obtained by centrifugation at $14{,}500 \times g$ for 30 min after incubation on ice for 30 min, and dialyzed against 20 mM Tris–HCl, pH 8.0 for 20 h.

Two-dimensional gel electrophoresis (2-DE)

For the first dimension, pH 3–10 immobilized pH gradient (IPG) gel strips (13 cm; Amersham Pharmacia Biotech, Cologno Monzese, Italy) were rehydrated overnight in a rehydration solution containing 100 μg of protein sample in an IPGphor strip holder covered with cover fluid. Isoelectric focusing was conducted at 20 °C using an IPGphor Isoelectric Focusing System (Amersham Pharmacia Biotech) (Shim et al., 2005). Prior to the 2-DE, the IPG gel strips were equilibrated and the second-dimensional separation was carried out on 12% SDS-PAGE gel (16×20 cm) without stacking gel at 4 °C. The IPG strips were embedded on top of the gel with 1% agarose. Electrophoresis was carried out at 60 mA/gel for 6 h until the bromophenol blue reached the bottom of the gel.

Western blot

Total proteins were separated by SDS-PAGE and transferred onto Immobilon-P membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 3%-skimmed milk, and then incubated with antibodies against caspase-3, IκBα IL-18, Fas, FADD, FasL, IκBα, Bad, Bcl-xL, cytochrome c, and α-tubulin. Immunocomplexes were detected by subsequent incubation with appropriate horseradish peroxidase-conjugated secondary IgG antibodies, and with enhanced chemiluminescence according to the manufacturer's protocol (Amersham Pharmacia Bioscience).

2-DE gel membrane was probed with rabbit anti-catalase antibody diluted 1:5000 in 3% skimmed milk, followed by an alkaline phosphatase-conjugated anti-rabbit antibody, and visualization was achieved with NBT:BCIP substrate kit (Bio-Rad Laboratories, Hercules, CA, USA).

Electrophoretic mobility shift assay

Nuclear extracts were prepared as described previously (Shim et al., 2005). Double-stranded deoxyoligonucleotide corresponding to NF-κB-responsive elements (Promega, Madison, USA) were end-labeled with [γ-^{32}P]dATP using T4 kinase (Takara, Tokyo, Japan). Nuclear extracts (8 μg) were incubated with poly (dI-dC) and ^{32}P-labeled DNA probe in a binding buffer (100 mM KCl, 30 mM Hepes, 1.5 mM $MgCl_2$, 0.3 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 1 mM PMSF, 1 μg/ml of aprotinin, and 1 μg/ml of leupeptin) for 15 min. Probes bound to NF-κB proteins were separated from the free probes using 5% polyacrylamide gel

to determine the DNA binding activity. Following electrophoresis, the gel was dried and subjected to autoradiography.

Preparation and transient transfection with E7 vector

E7 antisense (AS) plasmids were prepared as described previously (Shim et al., 2005). The pTarget vector and pTarget vector containing E7 reverse orientation were transfected into HaCaT/E7 cells using LipofectAMINE 2000 reagent as described earlier (Cho et al., 2001).

DCFH-DA assay

The ROS scavenging effect of E7 was measured using the oxidant sensitive fluorescent probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA). HaCaT/mock, HaCaT/E7, or HaCaT/E7 cells transfected with E7(AS) (1×10^6 cells) were preincubated with PBS in the presence of 20 μM DCFH-DA for 15 min at 37 °C. After stimulation with 250 μM H_2O_2 for 10 min, the relative green DCF fluorescence within live cells was measured using a flow cytometer (FACS Calibur, Becton Dickinson, Rutherford, NJ, USA).

IL-18 ELISA

Cells were treated with 0.5 and 1 mM of H_2O_2 for 16 h. The levels of IL-18 in the supernatants were subsequently measured with an ELISA kit (MBL) according to the manufacturer's instruction.

Statistical analysis

Data were presented as mean ± S.D. from one of three independent experiments performed in triplicate. Differences between means were assessed by one-way analysis of variance. Data were analyzed for statistical significance using ANOVA. The minimum level of significance was set at $p < 0.05$.

Results

Identification of E7-modulated proteins by 2-DE and Western blot analyses

Protein profiling was conducted in order to identify proteins modulated by E7 using 2-DE, silver staining, and matrix-assisted laser desorption/ionization time of flight (MALDI/TOF) mass spectrometry as previously described (Lee et al., 2005). In order to confirm the proteomics data, Western blot analysis was performed using polyclonal anti-catalase antibody. The HaCaT/E7 cells expressed higher amounts of various isoforms of catalase than the mock control did (Fig. 1).

Effect of E7-induced catalase on the formation of intracellular ROS

In order to find out whether the induction of catalase by E7 leads to lower levels of intracellular ROS (Shim et al., 2005), an ROS-sensitive DCFH-DA probe was used to measure intracellular ROS levels, as previously described (Cho et al., 2000). When cells were treated with 250 μM H_2O_2 for 10 min, a marked increase in the fluorescence was evident. The ROS level was significantly decreased in HaCaT/E7 (Fig. 2b), regardless of the presence of 250 μM H_2O_2 (Fig. 2c), while the transient transfection of HaCaT/E7 with E7(AS) generated a significantly higher amount of ROS than was observed in the HaCaT/E7 cells (Fig. 2e, f).

Expression of IL-18 in H_2O_2 treated HaCaT/E7

IL-18, a member of the IL-1 family, acts as a proinflammatory cytokine. It has been reported that IL-18 is inversely correlated with the viral gene expression transcriptionally in HPV 16 E7 immortalized cell lines (Cho et al., 2001). In this study, IL-18 expression was also down-regulated by human papillomavirus type 16 E7 oncogene (Fig. 3). Moreover, IL-18 expression is regulated by

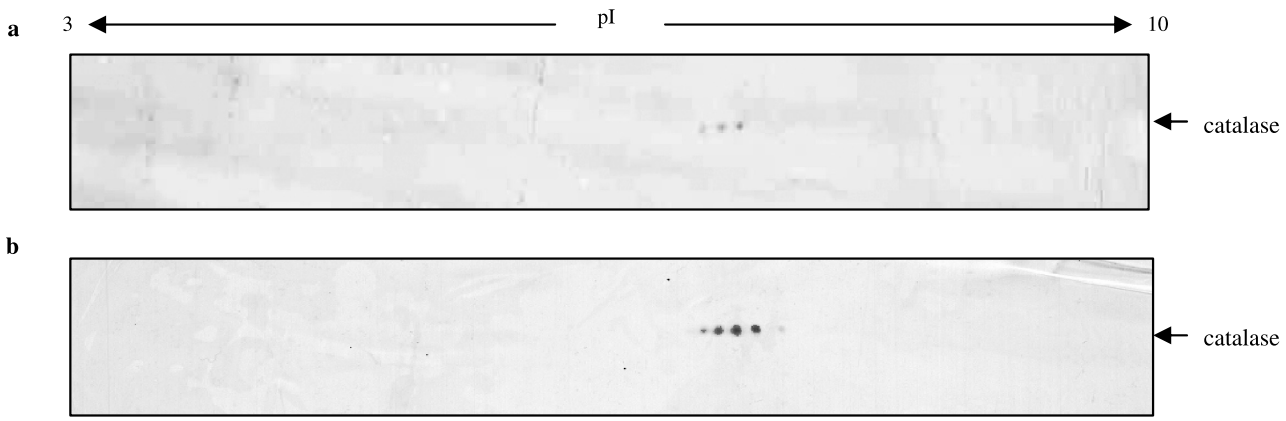

Fig. 1. 2-DE and Western blot analyses of HaCaT cells that stably express either mock (**a**) or E7 oncogene (**b**). Two hundred microgram of total cellular extract was separated using the isoelectric focusing method (immobiline gel range from pI 3 to 10) and second dimension is on 12% SDS-PAGE gel and visualized by immunoblotting with catalase specific antibody

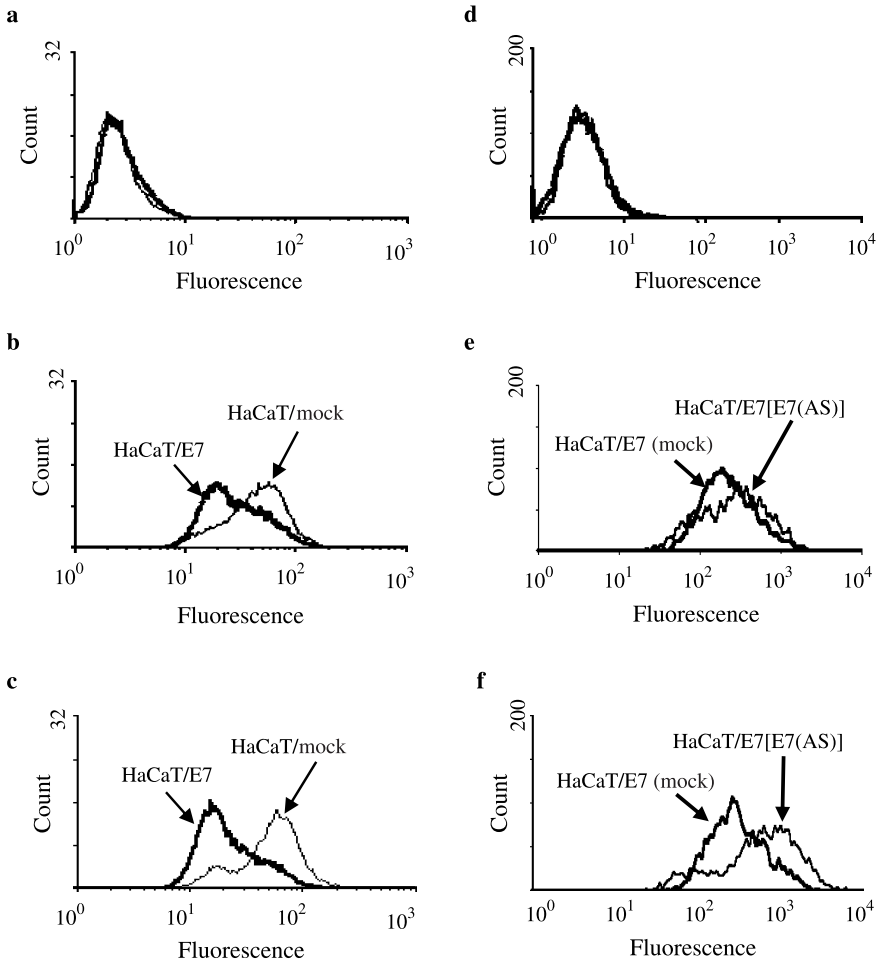

Fig. 2. Determination of intracellular ROS formation. HaCaT/mock, HaCaT/E7, or HaCaT/ E7 cells transfected with E7(AS) (1×10^6 cells) were preincubated with PBS in the absence (**a**, **d**) or presence (**b**, **c**, **e**, **f**) of DCFH-DA (20 μM) for 15 min at 37 °C. After exposure to 250 μM H_2O_2 (**c**, **f**), the relative green DCF fluorescence within living cells was measured with a flow cytometer as described in Materials and methods. These data represent one of three experiments. HaCaT/E7 (mock) represents HaCaT/E7 cells transfected with a control vector

oxidative stress (Chandrasekar et al., 2003). Hydrogen peroxide treatment downregulated the protein level of precursor IL-18 in HaCaT/E7 (Fig. 3a) and sequentially secretion of IL-18 into culture media was also reduced (Fig. 3b).

Specific effect of E7 on Fas receptor- and mitochondrial-mediated apoptosis by H_2O_2

The cytokine IL-18 possesses pleiotropic biological properties such as activation of tumor cell death via the Fas-FasL pathway (Dao et al., 1996) and Bcl-xL expression. In addition, it down-regulates the anti-apoptotic Bcl-2 and Bcl-xL gene expression, and activates caspases-8, -3, and -9 (Chandrasekar et al., 2004). As shown in Fig. 4, the levels of Fas protein were decreased by H_2O_2 in HaCaT/E7, whereas there were no perceptible changes of FasL in HaCaT/mock and HaCaT/E7. A high dose of H_2O_2 boosted Bcl-xL expression and concomitantly suppressed the expression of Bad protein in HaCaT/E7 (Fig. 4), supporting that a decrease in Bax translocation to mitochondria led to the altered mitochondrial membrane potential, reduced cytochrome c release and subsequent inactivation of caspase-3 in HaCaT/E7 cells (Shim et al., 2005).

Effect of NF-κB in H_2O_2-treated HaCaT/E7

NF-κB is known as an anti-apoptotic transcription factor by regulating several apoptosis-related proteins (Nakayama et al., 2001). To elucidate the involvement of NF-κB in modulating E7-induced anti-apoptotic phenomenon, Western blot analysis and gel-shift assay were performed. As shown in Fig. 5a, IκBα was partially degraded in HaCaT/E7 compared with HaCaT/mock, and the degradation was more prominent in the presence of 500 or 1000 μM of H_2O_2. Correspondingly, the DNA binding activity of NF-κB was highly induced in HaCaT/E7 in the absence or presence of 500 or 1000 μM of H_2O_2, suggesting that E7 induces IκBα degradation for NF-κB activation (Fig. 5).

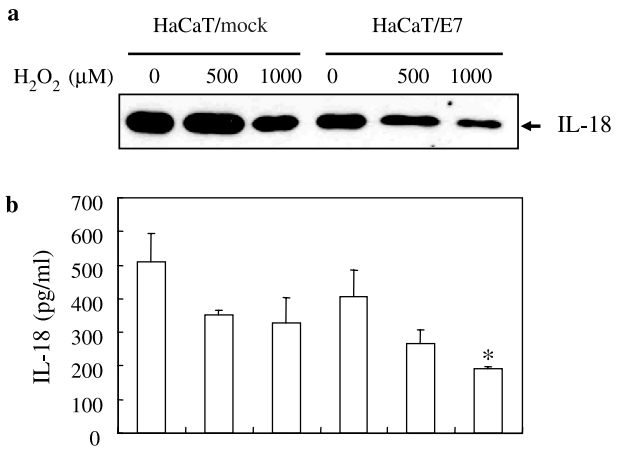

Fig. 3. Effect of hydrogen peroxide on the expression of IL-18 on HaCaT/E7. **a** IL-18 expression was determined by Western blot anlysis as described in Materials and methods. Twenty microgram of cellular extracts per lane was separated on a 12% SDS-PAGE gel as described in Materials and methods. Equal loading and protein transfer were confirmed by incubating the same membrane with anti-α-tubulin antibody. **b** The secreted IL-18 in H_2O_2-treated HaCaT/mock and HaCaT/E7 was detected with an IL-18 ELISA kit. Cells were treated with increasing concentrations of H_2O_2 for 16 h. IL-18 level in the supernatants was subsequently assessed by an ELISA according to the manufacturer's instruction. The optical density (O.D.) of each well was then measured at 450 nm using a microplate reader. The concentration of human IL-18 was calibrated by a standard curve

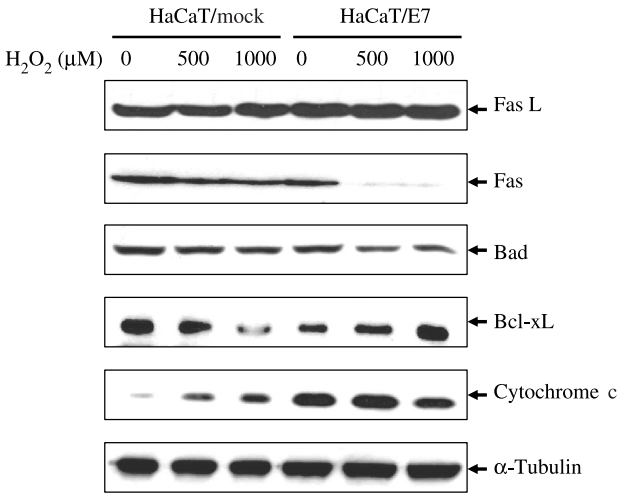

Fig. 4. The effect of E7 on receptor mediated apoptosis of HaCaT keratinocytes induced by H_2O_2. Fifty microgram of cellular extract per lane was separated on a 12% SDS-PAGE gel as described in Materials and methods. Equal loading and transfer of proteins was confirmed by incubating the same membrane with anti-α-tubulin antibody

Discussion

Many research groups have reported that viral E7 oncogene are selectively retained and expressed in carcinoma cells infected with human papillomavirus type 16 in order to enable keratinocytes to immortalize and transform via

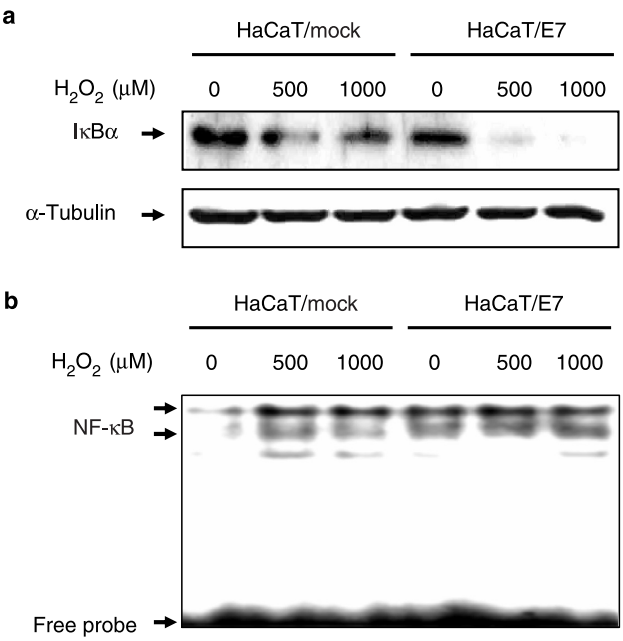

Fig. 5. Induction of IκBα degradation and activation of NF-κB by E7. **a** Western blot analysis of IκBα degradation. Cytosolic extracts (30 μg) per lane were separated on a 12% SDS-PAGE gel and then subjected to Western blot analysis using an anti-IκBα antibody. **b** Gel-shift assay. To examine the activation of NF-κB binding, nuclear extracts were obtained from cells treated with different doses (0, 500, and 1000 μM) of H_2O_2. The nuclear extracts were loaded onto 5% polyacrymide gel and probed by γ-^{32}p labeled-NF-κB. Specific binding of nuclear proteins to NF-κB responsive element were analyzed by electophoretic mobility shift assay

various pathways. The present study was performed to investigate the proteins involved in the immune escape and oncogenesis in oncogene-transfected cells as a model system. By using proteomics, we identified antioxidants such as catalase and Prx 2 that were induced by E7 oncogene. A catalase is also known to have anti-oxidant effects on various oxidative stimuli, such as hydrogen peroxide. Recently, it has been reported that HPV type 16 E7 gene protects against apoptotic and necrotic death induced by hydrogen peroxide (Lee et al., 1998, 2001). However, it has not yet been published that HPV 16 E7 is linked to anti-oxidant enzymes to prevent against oxidative stress (Lee et al., 2001). Therefore, we focused on the anti-oxidant enzymes in order to investigate whether E7 would make keratinocytes adaptive to the oxidative stress. HaCaT/E7 cells produced lower quantities of intracellular ROS, and appeared to be more resistant to H_2O_2-induced cell death. In order to investigate how E7 oncogene would modulate oxidative stress-induced cell death at high concentrations of H_2O_2 (500 μM–1 mM), we examined the effect of E7 oncogene on receptor mediated apoptosis (Fas-FasL pathway), cell death via the mitochondrial pathway and modulation of apoptosis relating factors (Bcl-xL, Bad,

cytochrome c). The increased catalase prevented cell death via the receptor-mediated apoptotic pathway as well as via the mitochondrial pathway. HaCaT/E7 exposed to H_2O_2 exhibited lower expression of IL-18, suggesting that H_2O_2 treatment led to the down-regulation of precursor IL-18, and reduction of mature IL-18 secretion into culture media, accompanied by lowered expression of surface Fas (Holler, 2002). In addition, the anti-apoptotic Bcl-xL gene involved in mitochondrial pathway was increased in a dose -dependent manner of hydrogen peroxide in HaCaT/E7 cells so that it could interfere with translocation of Bad to mitochondria, blocking downstream signaling to the mitochondrial membrane potential, cytochrome c release and the activation of caspase-3 (Shim et al., 2005). The initiator caspases appear to display some specificity, according to the type of apoptotic signal (Budihardjo et al., 1999; Earnshaw, 1999; Sun et al., 1999). Fas receptor-ligand interactions induce caspase-8 activation to trigger the downstream executioner caspase-3.

Western blot analysis exhibited that cleaved caspase-3 was detected significantly higher level in the H_2O_2-treated HaCaT/mock control cells than in the HaCaT/E7 cells (Shim et al., 2005). Apoptotic cell death is defined by the occurrence of a stereotype phenotype, including cell shrinkage, chromatin condensation, and nuclear fragmentation. To confirm that these changes were related to apoptosis, TUNEL assay was previously performed. TUNEL-positive cells were observed in the H_2O_2-treated HaCaT/mock control cells compared with the HaCaT/E7 cells, indicating that H_2O_2 treatment gives rise to apoptosis in HaCaT/mock control cells (Shim et al., 2005). NF-κB is also known as an anti-apoptotic factor via regulation of various proteins involved in apoptotic process, suggesting the possibility of its involvement in protective role of E7 against H_2O_2 – mediated cell damage. While IκBα was degraded, NF-κB activity was increased in HaCaT/E7 compared with HaCaT/mock in the presence of 500 or 1000 μM of H_2O_2. It turned out to be that E7 alleviated H_2O_2-mediated apoptotic cell death.

Taken together these results, it is possible to suggest that the HPV 16 E7 oncogene renders HaCaT/E7 cells resistant to ROS-induced cell injury, probably via the modulation of antioxidant enzyme catalase. In addition, attenuation of Fas-mediated apoptosis by H_2O_2 was attained through the inhibition of mitochondrial apoptotic pathway.

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References

Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281: 1322–1326

Boukamp P, Petrussevska R, Breitkreutz D, Hornung J, Markham A, Fusenig N (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol 106: 761–771

Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15: 269–290

Chandrasekar B, Colston JT, de la Rosa SD, Rao PP, Freeman GL (2003) TNF-alpha and H_2O_2 induce IL-18 and IL-18R beta expression in cardiomyocytes via NF-kappa B activation. Biochem Biophys Res Commun 303: 1152–1158

Chandrasekar B, Vemula K, Surabhi RM, Li-Weber M, Owen-Schaub LB, Jensen LE, Mummidi S (2004) Activation of intrinsic and extrinsic proapoptotic signaling pathways in interleukin-18-mediated human cardiac endothelial cell death. J Biol Chem 279: 20221–20233

Chao DT, Korsmeyer SJ (1998) BCL-2 family: regulators of cell death. Annu Rev Immunol 16: 395–419

Cho K, Yun C, Yoon D, Cho Y, Rimbach G, Packer L, Chung A (2000) Effect of bioflavonoids extracted from the bark of Pinus maritima on proinflammatory cytokine interleukin-1 production in lipopolysaccharide-stimulated RAW 264.7. Toxicol Appl Pharmacol 168: 64–71

Cho YS, Kang JW, Cho M, Cho CW, Lee S, Choe YK, Kim Y, Choi I, Park SN, Kim S, Dinarello CA, Yoon DY (2001) Down modulation of IL-18 expression by human papillomavirus type 16 E6 oncogene via binding to IL-18. FEBS Lett 501: 139–145

Cullen JJ, Weydert C, Hinkhouse MM, Ritchie J, Domann FE, Spitz D, Oberley LW (2003) The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. Cancer Res 63: 1297–1303

Dao T, Ohashi K, Kayano T, Kurimoto M, Okamura H (1996) Interferon-gamma-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell Immunol 173: 230–235

Earnshaw WC (1999) Apoptosis. A cellular poison cupboard. Nature 397: 387–389

Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN (1989) Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood 73: 334–339

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281: 1309–1312

Halbert C, Demers G, Galloway D (1991) The E7 gene of human papillomavirus type 16 is sufficient for immortalization of human epithelial cells. J Virol 65: 473–478

Hale AJ, Smith CA, Sutherland LC, Stoneman VE, Longthorne V, Culhane AC, Williams GT (1996) Apoptosis: molecular regulation of cell death. Eur J Biochem 237: 884

Holler E (2002) Cytokines, viruses, and graft-versus-host disease. Curr Opin Hematol 9: 479–484

Kahl R, Kampkotter A, Watjen W, Chovolou Y (2004) Antioxidant enzymes and apoptosis. Drug Metab Rev 36: 747–762

Kaufmann SH (1989) Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. Cancer Res 49: 5870–5878

Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG (1993) Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res 53: 3976–3985

Lee JE, Kim CY, Giaccia AJ, Giffard RG (1998) The E6 and E7 genes of human papilloma virus-type 16 protect primary astrocyte cultures from injury. Brain Res 795: 10–16

Lee KA, Kang JW, Shim JH, Kho CW, Park SG, Lee HG, Paik SG, Lim JS, Yoon DY (2005) Protein profiling and identification of modulators regulated by human papillomavirus 16 E7 oncogene in HaCaT keratinocytes by proteomics. Gynecol Oncol 99: 142–152

Lee WT, Lee JE, Lee SH, Jang HS, Giffard RG, Park KA (2001) Human papilloma virus type 16 E7 genes protect astrocytes against apoptotic and necrotic death induced by hydrogen peroxide. Yonsei Med J 42: 471–479

Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146: 3–15

Nakayama H, Ikebe T, Beppu M, Shirasuna K (2001) High expression levels of nuclear factor kappaB, IkappaB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. Cancer 92: 3037–3044

Parman T, Wiley MJ, Wells PG (1999) Free radical mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. Nat Med 5: 582–585

Reznikoff CA, Belair C, Savelieva E, Zhai Y, Pfeifer K, Yeager T, Thompson KJ, DeVries S, Bindley C, Newton MA, et al. (1994) Long-term genome stability and minimal genotypic and phenotypic alterations in HPV16 E7-, but not E6-, immortalized human uroepithelial cells. Genes Dev 8: 2227–2240

Shim JH, Cho KJ, Lee KA, Kim SH, Myung PK, Choe YK, Yoon DY (2005) E7-expressing HaCaT keratinocyte cells are resistant to oxidative stress-induced cell death via the induction of catalase. Proteomics 5: 2112–2122

Song S, Liem A, Miller JA, Lambert PF (2000) Human papillomavirus types 16 E6 and E7 contribute differently to carcinogenesis. J Virol 267: 141–150

Sun SY, Yue P, Wu GS, El-Deiry WS, Shroot B, Hong WK, Lotan R (1999) Implication of p53 in growth arrest and apoptosis induced by the synthetic retinoid CD437 in human lung cancer cells. Cancer Res 59: 2829–2833

Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456–1462

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