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XPC deficiency is related to APE1 and OGG1 expression and function

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ABSTRACT

Oxidative DNA damage is considered to be a major cause of neurodegeneration and internal tumors observed in syndromes that result from nucleotide excision repair (NER) deficiencies, such as Xeroderma Pigmentosum (XP) and Cockayne Syndrome (CS). Recent evidence has shown that NER aids in removing oxidized DNA damage and may interact with base excision repair (BER) enzymes. Here, we investigated APE1 and OGG1 expression, localization and activity after oxidative stress in XPC-deficient cells. The endogenous APE1 and OGG1 mRNA levels were lower in XPC-deficient fibroblasts. However, XPC-deficient cells did not show hypersensitivity to oxidative stress compared with NER-proficient cells. To confirm the impact of an XPC deficiency in regulating APE1 and OGG1 expression and activity, we established an XPC-complemented cell line. Although the XPC complementation was only partial and transient, the transfected cells exhibited greater OGG1 expression and activity compared with XPC-deficient cells. However, the APE1 expression and activity did not significantly change. Furthermore, we observed a physical interaction between the XPC and APE1 proteins. Together, the results indicate that the responses of XPC-deficient cells under oxidative stress may not only be associated with NER deficiency *per se* but may also include new XPC functions in regulating BER proteins.

1. Introduction

Base oxidation is the main DNA damage that results from a reactive oxygen species (ROS) attack. Guanine residues are the most common targets and 7,8-dihydro-8-oxodeoxyguanine (8-oxodG) is one of the most frequent lesions observed in cells [1,2]. Base excision repair (BER) is the main DNA repair pathway for oxidized DNA lesions. Although this pathway has been known for a long time, recent studies suggest that BER is more complex than initially described [1,2].

Certain evidence has shown that the nucleotide excision repair (NER) pathway is involved in repairing oxidized DNA damage [3]. The importance of NER is highlighted by different human syndromes, such as xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD), which are caused by NER gene mutations. Although the UV sensitivity and/or cancer predisposition of XP, CS and TTD patients can be explained by NER defect, other phenotypes (including neurological and developmental disorders) are not attributed to a UV lesion repair deficiency [4–7]. *XPC* and *XPA* gene mutations are associated with the highest tumor frequency in XP patients. In contrast, CS patients show severe neurodegeneration but low tumor frequency. The highest frequency and more severe neurological abnormality cases were observed among patients with *XPA* or *CSB* gene mutations, and studies provide support for accumulated oxidized DNA damage and

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mitochondrial dysfunctions in these backgrounds. In contrast, XPC patients do not develop neurodegeneration [6,7].

An association between NER and BER proteins in oxidized DNA damage repair has been previously suggested. DíErrico et al. showed that the XPC protein is involved in repairing 8-oxodG by stimulating OGG1 catalytic activity [8]. More recent data indicate that XPC participates in repairing oxidized DNA lesions [9]. CSB protein involvement in oxidized lesion repair has also been indicated [10–12]. Moreover, studies show that CSB stimulates NEIL1 DNA glycosylase repair activity [13] and regulates OGG1 expression levels [14]. Wong et al. also demonstrated that the CSB protein stimulates the AP site incision activity by APE1 [15].

APE1 is the main mammalian AP endonuclease and is an essential protein that regulates cellular responses to oxidative stress [16]. APE1 loss causes embryonic lethality in mice and apoptotic cell death in cultured cells [17]. This protein also participates in redox regulation of many transcription factors, such as NF-κB, Egr-1 and AP-1, to control different cellular processes [18]. APE1 subcellular localization is mainly in the nucleus, but it has also been observed in mitochondria and in the endoplasmic reticulum. Cytoplasmic localization has been observed in cells with high metabolic or proliferative rates as well as in several tumors, and it correlates with a poor prognosis [19]. APE1 accumulation in mitochondria enhances mtDNA repair capacity and cell survival after oxidative stress, which suggests that APE1 plays a role in maintenance of mtDNA integrity [20].

To better understand the interplay between NER and BER pathways, we investigated whether APE1 and OGG1 expression, activity and cell localization are affected in XPC-deficient cells under oxidative stress and whether such effects are associated with certain phenotypes observed in XP cells. The results suggest that XPC deficiency promotes a substantial disturbance on the APE1 and OGG1 expression as well as OGG1 activity. We suggest that XPC, APE1 and OGG1 may act collectively to promote oxidized lesion repair and regulate cellular responses to oxidative stress.

2. Materials and methods

2.1. Cell lines, culture conditions and DNA transfection

SV-40-immortalized (XP4PA-SV) and primary (AS189) human fibroblasts derived from XPC patients were used. DNA repair-proficient SV-40 immortalized (MRC5-SV) and primary fibroblasts (AS405 and GM00969) were used as controls. A description of each cell line [21–25] is provided in Table 1.

The cells were routinely grown at 37 °C and 5% CO_2 in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and antibiotics (0.1 mg/mL penicillin and 0.1 mg/mL streptomycin; Invitrogen).

The plasmid that coded for XPC-GFP and geneticin resistance gene (G418) was kindly provided by Dr. Emilie Renaud (from the Laboratoire de Génétique de la Radiosensibilité, Institut de Radiobiologie Cellulaire et Moléculaire, CEA, Fontenay-aux-Roses, France). Stable transfections were performed using LipoFectamine 2,000 (Life Technologies) in accordance with the manufacturer's instructions. Stable transfectants were selected in cultured media containing 800 μg/mL G418 and maintained in 400 μg/mL G418.

The plasmids pMDLg/pRRE, pMD2.G and pRSV/REV as well as a non-targeting control (shCOO2, SIGMA) or shRNA XPC (SIGMA, TRCN0000296565) were transfected into HEK293T cells using polyethylenimine. The virus containing supernatant was collected, filtered through a 0.22 μM filter and concentrated by ultracentrifugation (21.000 rpm for 2 h at 4 °C). Three days after transduction into GM00969 (CORIELL, Camden, New Jersey, USA) primary fibroblasts, the cells were selected using puromycin. The XPC and APE1 expression levels were evaluated using qPCR as described below.

2.1.1. Real-time PCR analysis (quantitative PCR)

The relative expression levels of APE1, XPC and OGG1 were determined using quantitative PCR experiments. Briefly, total RNA samples were obtained from NER-proficient and NER-deficient cell lines using the IllustraRNAspin Mini RNA Isolation Kit (GE Healthcare, Little Chalfont, United Kingdom), treated with DNAse I, and then used as a template for cDNA synthesis with random hexamers using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). For real-time PCR, a quantity of cDNA that corresponded to 10 ng of input RNA was used in each reaction. The reactions were performed using SYBR Green PCR Master Mix (Applied Biosystems) and analyzed in the StepOne Real-Time PCR System (Applied Biosystems). The relative expression levels were calculated using the GAPDH gene as the endogenous control. We used primer sequences from previous reports [26–30].

The following thermal cycling conditions were used: denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and then annealing and extension at 60 °C for 1 min. The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [31] considering the amplification efficiencies of the individual genes analyzed. A melting curve was created using the melting curve program in the PCR instrument to confirm the presence of a single PCR product.

2.1.2. Cell viability assays

Approximately 10^6 cells were grown in 100 mm Petri dishes for 18 h. The cells were rinsed once with a phosphate-buffered saline solution (PBS) and treated with H_2O_2 (Merck, Darmstadt, Germany) in DMEM without FBS for 20 min at 37 °C. For the riboflavin treatment, the cells were incubated with riboflavin in α-MEM medium (Invitrogen) for 30 min and then illuminated for an additional 30 min by placing the dishes on a 0.2 cm-thick glass surface sitting 10 cm above two fluorescent lamps (daylight lamps; Osram 15W; emission: 400–700 nm). After a H_2O_2 or riboflavin treatment, the cells were rinsed with PBS and incubated in supplemented DMEM for an additional 48 h.

Cellular viability was analyzed using a trypan blue dye exclusion assay as previously described [32]. Briefly, the cells were rinsed twice with PBS and harvested. Next, equal volumes of the cell suspension and 0.4% (w/v) trypan blue (Invitrogen) were mixed, and the cells were counted using a phase-contrast microscope and hemocytometer. Cell survival was estimated as the ratio of live cells and total cell number for three independent experiments.

For the clonogenic assay, approximately 1,000 cells were plated in 60 mm dishes and incubated for 14–16 h. The cells were then treated with methylene blue (MB) (Merck) in α-MEM with 5% FBS (Invitrogen) for 1 h at 37 °C; they were then rinsed with PBS and illuminated with visible light in α-MEM with 5% FBS for 20 min. After photoactivation, the cells were incubated in supplemented DMEM for 12–15 days. The cells were fixed with 10% formaldehyde and stained with 1% crystal violet solution. Colonies with more than 15 cells were counted, and survival was expressed by the efficiency of colony formation in MB-treated plates relative to the non-treated samples.

Additionally, XPC-cell sensitivity to MB was also analyzed using the ViaCount assay. Briefly, 10^5 cells were seeded in 6-well plates and treated with MB as described. The cells were harvested 48 h later, and an aliquot that corresponded to ¼ of the cell suspension was rinsed with PBS and mixed with the ViaCount reagent (Merck Millipore, Billerica, MA, USA) to determine cell viability. The remaining cells were fixed with 70% ethanol to analyze the apoptosis profile by quantifying a sub-G1 population. Briefly, the fixed