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Enhancement of UVB-induced DNA damage repair after a chronic low-dose UVB pre-stimulation

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ABSTRACT

Absorption of solar ultraviolet (UV) radiation by DNA leads to the formation of the highly mutagenic cyclobutane pyrimidine dimer (CPD). The mutagenicity of CPD is caused, in part, by the fact that their recognition and repair by the nucleotide excision repair (NER) pathway is challenging and slow. It has been previously shown that a pre-stimulation with genotoxic agents improve NER efficiency of CPD, indicating a potential adaptive response of this repair pathway. We have pre-treated human dermal fibroblasts with repeated subletal low doses of UVB (chronic low-dose of UVB; CLUV) to determine whether it could enhance NER capacity to repair CPD. Our results show that CLUV pre-treatment greatly enhances CPD repair but have little effect on the repair of another UV-induced bypirimidine photoproduct, the pyrimidine (6-4) pyrimidone photoproducts (6-4 PP). We have determined that the CLUV treatment activates p53 and we found an increase of *DDB2* and *XPC* gene expression. This is consistent with an increasing level of NER recognition proteins, DDB2 and XPC, we found concentrated at the chromatin. This study represents the first demonstration that chronic UVB exposure can stimulate NER pathway. Altogether, these results shed light on the potential adaptability of the NER by chronic UVB irradiation and the mechanisms involved.

1. Introduction

Exposure to solar radiations is involved in skin cancer initiation and progression [1,2]. More precisely, the ultraviolet type-B (UVB) component of sunlight induces highly mutagenic DNA damage responsible for UV signature mutations found in skin cancer [3,4]. Genotoxicity of UVB is mainly due to their direct absorption by DNA, leading to the formation of cyclobutane pyrimidine dimers (CPD), pyrimidine (6-4) pyrimidone photoproducts (6-4 PP) and Dewar valence isomer of the 6-4 PP [5]. CPD are the most mutagenic UV-induced DNA lesion for humans. They are abundant (almost 70% of UVB-induced DNA damage) [6] and their recognition by the repair pathway is challenging and slow [7]. On the contrary, since 6-4 PP are repaired much faster than CPD, they can be considered less mutagenic in human cells [3,8].

To counteract the deleterious and mutagenic effect of UVB-induced CPD, eukaryotic cells use a network of mechanisms called the DNA damage response pathway (DDR). The central role of DDR is to avoid cellular transformation and, to achieve it, DDR signals the presence of DNA lesions and activates different signaling pathways to removes or tolerates them [9]. Genes controlling the DDR response are frequently affected and disrupted in cancer [10]. One of the most important and central gene involved in many DDR pathways is the *TP53* tumor suppressor gene. More precisely, *TP53* product is involved in the control of cell cycle progression, apoptosis, and repair pathway [10–13] and a deficiency in p53 protein leads to a defective DDR [14].

The main pathway activated in the DDR is the DNA repair system. In human, the nucleotide excision repair (NER) is the only system able to repair UV-induced bipyrimidine photoproducts. NER pathway involved more than 30 proteins and its importance is well demonstrated by the fact that a deficiency in NER protein, such as in *Xeroderma Pigmentosum* (XP) patients, induces photosensitivity and an increase up to 1000-fold of skin cancer occurrence [15,16]. The protein p53 regulates the NER pathway by acting as a transcription factor for different NER-involved proteins or through direct interaction with DNA damage [17] and a lack of functional p53 leads to a defective NER repair [18,19].

In the NER pathway, DDB2 and XPC proteins are crucial in the recognition of bipyrimidine photoproducts [20–22]. Both proteins need to efficiently recognize the DNA lesion to avoid its conversion to mutation. It has been shown that p53 acts as a transcription factor for the genes coding for DDB2 and XPC [23,24]. DDB2 is the first protein translocating to the chromatin and to bind directly the lesion [25]. Among others, this binding causes the recruitment of XPC, which is a

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key factor for NER continuity and protein recruitment [21,22]. Cells lacking DDB2 are deficient in CPD repair, and have a reduced repair rate of 6-4 PP, which leads to an increased UV sensitivity [24,26,27].

DDR mechanisms involved in UV-induced cellular response, and especially in NER pathway, have been extensively studied after a single UVB exposure. We have previously shown that exposure to chronic low dose of UVB (CLUV) irradiation modulates genotoxic stress response [28]. In addition, pre-stimulation with ionizing radiation or quinacrin mustard improves NER of UV-induced CPD [29,30]. Ionizing radiation and quinacrine mustard induce DNA damage that are not normally repaired by NER but rather by recombination (strand breaks) and base excision repair (oxidized bases). This suggests that any genotoxic stress, independent of the NER pathway, potentially lead to an adaptability of the NER. Nevertheless, the potential adaptive response of the NER pathway after chronical UVB exposure is poorly considered.

In the present study, we used CLUV irradiation as a pre-treatment to determine whether it could influence the NER efficiency of CPD and 6-4 PP. We observed that the CLUV treatment leads to the accumulation of residual and unrepairable CPD. On the other hand, the CLUV pre-treatment greatly enhance CPD repair of newly generated CPD but have little effect on 6-4 PP repair. More precisely, the CLUV treatment activates p53, which corroborate with the increased level of DDB2 and XPC gene and proteins. We found more DDB2 and XPC recruited at chromatin bound, suggesting a more efficient CPD recognition by NER. Altogether, these results shed light on the potential adaptability of the NER by chronic irradiation and the mechanisms involved.

2. Materials and methods

2.1. Ethic statement

All experiments performed in this study were conducted in accordance with our institution's guidelines and the Declaration of Helsinki. The research protocols received approval by the Centre de Recherche du CHU de Québec (CRCHUQ) institutional committee for the protection of human subjects.

2.2. Cell culture

Normal human diploid fibroblasts (NHDF) were obtained by skin biopsy (mastectomy) and provided from 4 different healthy patients aged between 18–38 years old. Cells were cultured in Dulbecco's modified Eagles' medium (DMEM) (Corning cellgro) complemented with 10% FBS and 1% penicillin/streptomycin (Wisent, Qc, CA) at 37 °C, 5% CO_2.

2.3. UVB irradiation and CLUV treatment

NHDF were irradiated using RPR-3000 UVB lamps with a peak emission at 300 nm (Southern New England Ultraviolet Co.). A cellulose acetate sheet (Kodacel TA-407, clear 0.015 in.; Eastman-Kodak Co.) was used to filter out wavelengths below 295 nm [31].

Confluent cells were irradiated using 3 different conditions: a single irradiation of 400 J/m^2 of UVB, a CLUV treatment or a CLUV treatment followed by a single irradiation (corresponding to pre-stimulated cells). Briefly, the CLUV treatment was as follow: 75 J/m^2 of UVB every 12 h for 7.5 days (total 15 irradiations, 1125 J/m^2). After the CLUV treatment, cells were incubated for 12 h and harvested, or exposed to the single irradiation of 400 J/m^2 of UVB. For more details see [28]. Cells are then harvested between 0 and 24 h post-irradiation.

2.4. DNA damage and repair assay

Total DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen Germany) following the manufacturer's protocol with an additional RNase treatment. DNA concentration was evaluated using a spectrophotometer (NanoDrop 2000; Thermoscientific, MA, USA).

The repair assay was performed using the immune-slot-blot technique as previously described [33] using a mouse anti-CPD monoclonal antibody (Cosmo Bio Co., clone TDM-2) and a mouse anti-ssDNA monoclonal antibody (EMD Millipore, clone 16–19). Membranes analysis and quantification were performed with C-DiGit® Blot Scanner (LI-COR Biosciences).

NHDF from 3 different cell strains were used for this experiment, and the slot blot is performed at least twice for each NHDF cell strains. Significance level was defined for P-values ≤ 0.05, derivate from the two-tailed heteroscedastic Student's t-test.

2.5. RNA isolation and gene profiling assay

Total RNA was extracted using TRIzol® Reagent (Life Technologies, Canada) and RNA quantity and quality were assessed using a 2100 Bioanalyzer Instruments (Agilent Technologies. USA) according the manufacturer's guidelines. Gene expression profiling was performed for CLUV treated cells and un-irradiated cells using the One-color Microarray-Based Gene Expression Analysis guidelines as described previously [32]. Briefly, amplified cRNA were hybridized on a G4851A SurePrint G3 Human Ge 8 × 60 K array slide (Agilent Technologies, USA) and scanned on an Agilent SureScan Scanner. Gene expression was determined using Arraystar v4.1 software (DNASTAR). Statistical data analysis has been done using the multiarray analysis for background correction of the raw data values.

2.6. Quantitative real-time PCR analysis

cDNA was synthetize using the TaqMan® Reverse Transcription Reagent (Applied Biosystems, Roche, Canada) as described in [33]. Briefly. DDB2 and XPC mRNA level were quantitatively measured using the Rotor-Gene Q real-time thermocycler (Qiagen, Germany). QPCR reactions were achieved using the Brilliant III Ultra Fast SYBR Green Master Mix (Agilent Technologies, USA). The following primer sequences of DDB2 were used: Forward 5′CAT CAA AGG GAT TGG AGC TG and Reverse 5′CTA CTA GCA GAC ACA TCC AGG CTA. The XPC primers are as following: Forward 5′TGA CCT CAG GGA CTT TCC AAG and Reverse 5′AAT TCT TAT CTC CAC TGG CTT CAG. The sequences of GAPDH gene used were: Forward 5′AAG GTC GGA GTC AAC GGA T and Reverse 5′GGA AGA TGG TGA TGG GAT TTC. The qPCR cycles were as follow: 95 °C for 10 min followed by 40 cycles of 40 s at 95 °C, 40 s at 63 °C and 40 s at 72 °C. All samples were done in quadruplicate for each gene. Data analysis was based on the $2^{-\Delta\Delta Ct}$ method [34], using the threshold cycle (Ct). Un-irradiated controls (NoUV) was used as a baseline of gene expression (value = 1) and GAPDH as endogenous control. 4 different cell strains were used for this experiment.

2.7. Protein extraction and western blot

Proteins were isolated in 3 cellular fractions (cytosolic, nuclear, chromatin bound) as previously described [33]. Briefly, cells were resuspended 30 min at 4 °C in cytosolic lysis buffer (10 mM Tris pH 8, 0.34 M Sucrose, 3 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5% NP40) with 40 µl/mL of protease inhibitor (Complete EDTA free, Roche) and centrifuged (5 000 rpm, 2 min). The supernatant was kept as the cytosolic fraction. Pellets containing nuclear and chromatin bound fractions were resuspended in 2.5 vol of nuclear lysis buffer (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 1 mM EDTA, 150 mM KCl, 0.1% NP40, 1 mM DTT, 10% glycerol with 40 µl/mL of protease inhibitor). Nuclei were broken using syringe and needle (21G), following a centrifugation (14,000 rpm, 30 min). Supernatant was isolated as the nuclear fraction. The residual pellet containing the chromatin fraction was resuspended for 1 h, at 4 °C in 2 vol of nuclease incubation buffer (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 150 mM KCl, 10% glycerol and 0.15 units/µl benzonase) and centrifuged (14,000 rpm, 3 min) to get rid