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Rapid inactivation and proteasome-mediated degradation of OGG1 contribute to the synergistic effect of hyperthermia on genotoxic treatments

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ABSTRACT

Inhibition of DNA repair has been proposed as a mechanism underlying heat-induced sensitization of tumour cells to some anticancer treatments. Base excision repair (BER) constitutes the main pathway for the repair of DNA lesions induced by oxidizing or alkylating agents. Here, we report that mild hyperthermia, without toxic consequences per se, affects cellular DNA glycosylase activities, thus impairing BER. Exposure of cells to mild hyperthermia leads to a rapid and selective inactivation of OGG1 (8-oxoguanine DNA glycosylase) associated with the relocalisation of the protein into a detergent-resistant cellular fraction. Following its inactivation, OGG1 is ubiquitinated and directed to proteasome-mediated degradation, through a CHIP (C-terminus of HSC70-interacting protein) E3 ligase-mediated process. Moreover, the residual OGG1 accumulates in the perinuclear region leading to further depletion from the nucleus. As a consequence, HeLa cells subjected to hyperthermia and exposed to a genotoxic treatment have a reduced capacity to repair OGG1 cognate base lesions and an enhanced cell growth defect. The partial alleviation of this response by OGG1 overexpression indicates that heat-induced glycosylase inactivation contributes to the synergistic effect of hyperthermia on genotoxic treatments. Taken together, our results suggest that OGG1 inhibition contributes to heat-induced chemosensitisation of cells and could lay the basis for new anticancer therapeutic protocols that include hyperthermia.

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1. Introduction

Mild hyperthermia is defined as the exposure to non-lethal temperatures higher than the physiological one. Several reports established that exposure to mild hyperthermia sensitizes cancer cells to chemotherapy and radiotherapy, hence hyperthermia has been put under evaluation as adjuvant in anticancer therapeutic procedures, showing promising results in phases II–III trials [1–3]. Even if the underlying molecular mechanisms have not been comprehensively characterized yet, several evidences indicated that hyperthermia affects DNA repair by homologous recombination (HR), thus sensitizing cells to agents generating DNA double strand breaks (DSBs) [4–7]. Nevertheless, other pathways are also involved in repairing lesions induced by the anticancer agents whose activity is potentiated by hyperthermia. For example, up to 80% of the DNA lesions induced by radiation are base modifications [8], thus recognized and processed by the DNA base excision repair (BER) pathway. Moreover, BER is the major DNA repair pathway involved in repairing damage induced by alkylating compounds [9], also frequently used in cancer therapy. For these reasons, the involvement of BER in heat-induced sensitization of cancer cells has been hypothesized [10], but experimental evidences are still lacking.

BER is a DNA repair pathway dealing with a large set of subtle, non-bulky base lesions including some that are cytotoxic if left unrepaired, such as 5′,6′-dihydroxydihydrothymine (thymine glycol) [11] or imidazole ring-opened 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (FaPy) lesions [12]. Also 8-oxo-7,8-dihydroguanine (8-oxoG), which is one of the most common purine lesions and is highly mutagenic [13,14], may trigger cell death [15,16] or inhibit cancer cell growth [17]. DNA glycosylases are the initiating enzymes of the BER pathway [18]. They recognize and excise the cognate damaged base on DNA, thus generating an apurinic/apyrimidinic (AP) site. The second step of the pathway is the incision of the DNA strand immediately upstream of the AP site by an AP endonuclease, APE1 in mammals. Alternatively, some bi-functional DNA glycosylases, such as 8-oxoguanine DNA glycosylase (OGG1) or endonuclease III-like 1 glycosylase (NTHL1) can cleave the AP site leaving a deoxyribose

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residue that is then excised by APE1. In BER's simplest version, the resulting gap is further filled by DNA polymerase β and sealed by DNA ligase III [19].

BER has been shown to confer resistance against anticancer agents [20,21]. For this reason it was proposed that BER inhibition, in association with DNA-damaging agents, could be exploited in anticancer therapy [22,23]. APE1 has been the most studied target for the development of small molecule BER inhibitors [24,25]. APE1 inhibitors were also found to cause cell death of DSB repair-deficient cells [26], similarly to what was already observed for PARP-1 inhibitors [27]. The use of DNA polymerase β inhibitors has also been proposed [28–30]. The strategy of interfering with the initial step of BER has never been addressed, in spite the fact that several evidences point to DNA glycosylases as having a role in protecting cells from anticancer treatments. In particular, OGG1, which is the functional analogue of bacterial formamidopyrimidine DNA glycosylase (Fpg) as it is responsible for 8-oxoG and FaPy lesions removal in eukaryotic cells [31–36], may represent a novel target in anticancer therapy. First, OGG1 or Fpg overexpression confers resistance to several alkylating agents, including aziridine [37], N,N′,N″-triethylenethiophosphoramide (thioTEPA) [38], 1,3-N,N′-bis(2-chloroethyl)-N-nitrosourea (BCNU) and mafosfamide [39], as well as to platinum compounds [40]. Moreover, OGG1 activity is up-regulated in some cancers [41,42] and a strong correlation was found between high OGG1 expression and poor prognosis in acute myeloid leukaemia patients due to a reduction in cancer cell sensitivity to chemotherapeutic agents [43]. This latter observation is likely imputable to the increased effectiveness in repairing FaPy lesions they induce [38]. Therefore, strategies aiming at blocking or reducing OGG1 activity could be profitably implemented in combination with conventional anticancer therapy, especially in cancers displaying increased OGG1 activity.

Here we address the question of whether mild heat shock could interfere with BER. Since it was previously shown that heat shock does not inhibit APE1 [44] and affects only marginally DNA polymerase β activity [45], we investigated whether base lesions are efficiently recognized and processed by DNA glycosylases during hyperthermia. Our results show for the first time that hyperthermia impairs OGG1 activity and therefore cells' ability to repair OGG1 cognate lesions. Indeed, OGG1 is a heat-sensitive enzyme. Moreover, hyperthermia triggers OGG1 ubiquitination and targets the glycosylase to proteasome-mediated degradation. The data presented below suggest that heat-induced sensitization of cancer cells to genotoxic agents is due, at least in part, to impairment of the first step of BER.

2. Materials and methods

2.1. Cell culture, transfection and heat shock

HeLa cells (cervix cancer cell line) were obtained from the ATCC and SH-SY5Y cells (neuroblastoma cell line) were kindly provided by Marc Fontaine (Université de Rouen). Cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 10 μg/ml streptomycin sulfate (Invitrogen). HeLa cells overexpressing hOGG1-GFP were described before [46] and were grown in medium supplemented with 400 μg/ml geneticin (Invitrogen). A construct coding for OGG1-FLAG that has previously been described [47] was transiently transfected in HeLa cells using Lipofectamine 2000 reagent (Invitrogen) and following manufacturer instruction. Cells were treated 24 h after transfection. For CHIP silencing, a specific siRNA (Eurogentec), having the sequence GGAGCAGGGCAATCGTCTG, was used as already described before [48]. Briefly, the CHIP-specific siRNA or the siCONTROL RISC free (Dharmacon) together with the plasmid coding for OGG1-FLAG were co-transfected in cells using Lipofectamine 2000 reagent (Invitrogen) and following manufacturer's instructions. Cells were treated 48 h after co-transfection. Cells were cultured in a humidified incubator at 5% CO_2 and 37 °C and fed with fresh medium at 48-h intervals. For heat shock treatments, cells were kept in a humidified incubator at 5% CO_2 and 42 °C for the indicated times.

2.2. Protein extracts preparation, fractionation and immunoprecipitation

After treatment, cells were washed twice with ice-cold PBS, collected by scraping, centrifuged at 250 × g for 10 min at 4 °C and cell pellets were frozen at −80 °C. For DNA glycosylases and AP endonuclease activities assays, cell pellets containing about 5 million cells were resuspended in 150 μl of an ice-cold buffer containing 20 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1 mg/ml BSA and 100 mM sucrose supplemented with protease inhibitor cocktail (Sigma) and then sonicated using a BioRuptor sonicator (Diagenode) operated at 4 °C. 10 high-intensity 30-s sonication pulses were applied. Cell lysates were then centrifuged at 16,000 × g for 30 min at 4 °C, supernatant was collected and protein content was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories). For preparation of whole cell lysates, pellets containing about 5 million cells were resuspended in 300 μl of 2× Laemmli sample buffer (100 mM Tris–HCl pH 6.8, 20% glycerol, 40 mg/ml SDS, 0.02% bromophenol blue) containing (reducing) or not (non reducing) 200 mM DTT. Samples were sonicated, boiled for 5 min at 96 °C and loaded on SDS-PAGE. To prepare the detergent-soluble and the detergent-resistant protein fractions, cell pellets (about 5 million cells) were incubated for 10 min at 4 °C in 150 μl ice-cold CSK buffer (100 mM NaCl, 300 mM sucrose, 10 mM PIPES, pH 6.8, 3 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA) containing 0.5% Triton X-100 and protease inhibitors. After centrifugation at 5000 × g for 5 min, the supernatant (soluble proteins) was recovered as the detergent-soluble fraction and 50 μl of reducing 4× Laemmli sample buffer were added. Pellets were washed three times with 1 ml ice-cold CSK and centrifuged at 5000 × g for 5 min. The resulting pellets were resuspended in 200 μl of reducing 2× Laemmli sample buffer. Resuspended detergent-resistant samples were sonicated as described before, boiled and centrifuged for 10 min at 13,000 × g before loading on SDS-PAGE. For immunoprecipitation, EZview Red ANTI-FLAG M2 Affinity Gel (Sigma) was used. Protein extracts were prepared and immunoprecipitation was conducted as described [49].

2.3. DNA glycosylases and AP endonuclease activities assays

Three double-stranded 34-mer probes containing respectively a tetra-hydro-furan (THF), an 8-oxoG and a thymine glycol (Tg) were prepared. The oligonucleotides containing a tetra-hydro-furan (THF) or a 8-oxoG at position 16 were 5′-end labelled by Cy5 and were hybridized to the complementary oligonucleotides containing a cytosine opposite the lesion, yielding the THF:C and the 8-oxoG:C duplexes. The oligonucleotide containing a thymine glycol (Tg) at position 16 was labelled at the 5′ end using [γ-32P]ATP (3000 Ci/mmol; Perkin Elmer) and T4 polynucleotide kinase (New England Biolabs). The ^{32}P-labelled strand was hybridized to its complementary oligonucleotide containing an adenine (A) opposite to the lesion yielding the Tg:A duplex. Reactions were carried at 37 °C in a total volume of 14 μl, containing the specified amount of protein extract and 150 fmol of DNA probe. Compositions of reaction buffers were 22 mM Tris–HCl pH 7.4, 110 mM NaCl, 2.5 mM EDTA, 1 mg/ml BSA and 5% glycerol for Tg:A and 8-oxoG:C probes and 25 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mg/ml BSA, 1 mM $MgCl_2$ and 3% glycerol for THF:C probe. Reaction mixtures containing 8-oxoG:C probe were incubated at 37 °C for 1 h, then NaOH (0.1 N final concentration) was added, the mixture was further

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