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ScienceDirect
Journal of Photochemistry and Photobiology B: Biology 90 (2008) 8–16
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Only complete rejoining of DNA strand breaks after UVC allows K562 cell proliferation and DMSO induction of erythropoiesis

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Received 18 December 2006; received in revised form 10 May 2007; accepted 17 May 2007
Available online 23 October 2007

Abstract

DNA strand breaks are early intermediates of the repair of UVC-induced DNA damage, however, since they severely impair cellular activities, their presence should be limited in time. In this study, the effects of incomplete repair of UVC-induced DNA strand breaks are investigated on K562 cell growth and the induction of erythroid differentiation by addition of DMSO to the cell culture medium. The kinetics were followed after UV irradiation by single cell gel electrophoresis, and in total cell population by alkaline or neutral agarose gel electrophoresis. Shortly after exposure, an extensive fragmentation occurred in DNA; DNA double strand breaks were negatively correlated with recovery time for DNA integrity. DNA damage induced by UVC 9 J/m^2 rapidly triggered necrosis in a large fraction of irradiated K562 cells, and only 40% of treated cells resumed growth at a very low rate within 24 h of culture. The addition of DMSO to the culture medium of cells 15 min after UVC, when DNA strand break repair was not yet complete, produced apoptosis in >70% of surviving cells, as determined by TUNEL assay. Conversely, if DMSO was added when the resealing of DNA strand breaks was complete, surviving K562 cells retained full growth capacity, and their progeny underwent erythroid differentiation with normal levels of erythroid proteins, δ-aminolevulinic acid dehydrase and hemoglobin.

This study shows that the extent of DNA strand break repair influences cell proliferation and the DMSO induced erythroid program, and the same UVC dose can have opposite effects depending on cellular status.

Keywords: UVC; DNA repair intermediates; SSBs; DSBs; Differentiation; Apoptosis

1. Introduction

The consequence of DNA damage is quite diverse and may involve disturbed DNA metabolism, cell-cycle arrest, cell death and long-term effects such as carcinogenesis [1]. UV-induced lesions are representative of many other agents. With respect to DNA, cyclobutane pyrimidine dimers (CPDs) and the 6–4 photoproducts (6–4PPs) are the predominant lesions caused by short-wavelength (254 nm) UV light (UVC) [2], in humans, these DNA damages are removed by the nucleotide excision repair (NER). This system has been characterized and many aspects of this repair at the molecular level are understood, including the proteins involved in recognition, elimination and re-synthesis [3,4].

DNA strand breakage is a major threat to genetic stability, the most common are single-strand breaks (SSBs), and double-strand breaks (DSBs). SSBs are present as intermediates during replication, in Okazaki fragments, and cell differentiation, these physiological SSBs are not harmful to cells if properly repaired. However, SSBs result as enzymatically produced intermediates in Nucleotide Excision Repair (NER) activated in response to the UV-induced CPDs and 6–4PPs UV. Replication forks stall or collapse at DNA lesions, and these events have often been associated with recombination and chromosomal rearrangements. Stalled forks generate SSBs that

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doi:10.1016/j.jphotobiol.2007.05.008

activate the replication checkpoint, which in turn functions to protect the stability of the fork until the replication can resume [5].

DSBs are some of the more detrimental DNA lesions as they disrupt both DNA strands. DSBs can be directly induced by extrinsic agents as reactive oxygen species, ionizing radiation, and chemicals that generate reactive oxygen species. Moreover, the majority of DSBs are produced enzymatically, and arise in DNA as intermediates during mitotic and meiotic recombination, DNA replication, transposition of certain mobile elements, transduction, transformation, and V (D) J recombination in the vertebrate immune system, respectively. The action of restriction endonucleases and topoisomerases also generates DSBs [6]. In eukaryotes, homologous recombination (HR) typically uses the sister chromatid or homolog as a template to repair DSBs in a largely error-free way, whereas non-homologous recombination (NHEJ) directly joins DNA ends in a template-independent manner that, depending on the exact DNA end structure, leads to error-prone DSB repair [4]. After UV irradiation, most cellular activities are temporarily suspended to repair damage to DNA.

Even if they are activated immediately, DNA repair pathways require time to restore original sequence and DNA structure, and during this period normal DNA replication and transcription are modified to cope with specific repair proteins and unscheduled DNA synthesis.

In order to investigate how the induction of a differentiative process could be affected by the presence of DNA strand breaks, we studied, in UVC-irradiated human K562 cells, the effects of SSBs and DSBs on the switch of the erythroid pathway by dimethyl sulfoxide (DMSO). This compound is a polar/apolar inducer and its target is the plasma membrane [7]. In murine erythroleukemia cells has been shown that a cation-dependent modulation of DMSO activity affected early steps of cell commitment and signal transduction. In Mel cells an early event was the activation of a Ca^{2+} Mg^{2+}-dependent endonuclease before commitment to erythroid differentiation by DMSO [8].

DMSO can act as a scavenger of some free radicals (OH radicals) [7], and SSBs and DSBs are induced on DNA early after its addition to murine erythroleukemia cells [9].

While terminal differentiation of myeloid cells may result in apoptosis, it has been shown that differentiating myeloid cells can become resistant to various apoptotic stimuli [10]. This indicates that cellular differentiation can affect apoptosis sensitivity. Here, we report studies on DMSO effects on proliferation and erythropoiesis induction, in UVC irradiated K562 cells, in relation to the kinetics of DNA strand breaks rejoining. We show that there is a precise time window after UVC irradiation of replicating cells in which DNA strand breaks are not yet repaired and DMSO addition leads to cell death.

The relationship between apoptosis and differentiation appears interesting, especially in the possible use of differentiation therapy in combination with irradiation.

2. Materials and methods

2.1. Cell culture and irradiation conditions

The human leukemia K562 cells are widely used as model system for studies on erythropoiesis [11]. These cells were cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin and streptomycin (SIGMA) in a 5% CO_2 atmosphere at 37 °C. For UVC irradiation, exponentially growing cells were pelletted and rinsed with phosphate-buffered saline pH 7.0 (PBS), resuspended in 1 ml of PBS to a thin layer on a plastic dish (without lid) and irradiated with 254 nm UVC (Spectroline lamp) at 4.5 /m^2/s for 2 s (dose = intensity × time = 9 J/m^2) or 3 s (dose = 13.5 J/m^2), at 15 cm of height from the cell monolayer. In all subsequent steps, cells were always kept in the dark. Immediately after irradiation, K562 cells were rapidly pelletted and, within a few minutes (<5 min) of irradiation the cells were processed immediately, or were resuspended at the density of 5×10^4 cells/ml in 5 ml of their prewarmed medium for proliferation and differentiation studies. Cell viability was assessed by the Trypan blue exclusion method, by mixing (1 vol: 1 vol) Trypan blue (4% in PBS) and cells, and counting the number of cells that excluded the dye in a Burker chamber within 5 min of staining.

Erythroid differentiation was started by the addition of 1% DMSO to the culture medium of K562 cells, and the number of differentiated cells was assessed, after a week of cell incubation, using the benzidine test [12]. Hemoglobin was measured spectrophotometrically at 415 nm, in clear cell supernatants of lysed K562 cells that had been incubated for 7 days in a complete medium supplemented with 1% DMSO (induction medium).

Activity of δ-aminolevulinic acid dehydrase (ALA DH) was assessed in 1×10^7 cells, at day 4 of culture in the induction medium, and porphobilinogen was measured in cell lysates according to Sassa and Berstein [13]. The basal value of porphobilinogen in cells cultured without DMSO was subtracted from the value determined in the induced cells, and the obtained value was successively normalized for total proteins in the cell lysate. Protein content was assessed in the clear supernatant of lysed cells measuring OD at 280 nm.

2.2. Apoptosis detection by TUNEL

K562 cells were washed three times in PBS and adjusted to a density of 2×10^7 cells/ml. The cells were then transferred into a V-bottom 96-well microtiter plate (100 μl/well) and fixed in paraformaldehyde solution (4% in PBS, pH 7.4) for 1 h at room temperature, on a shaker to avoid extensive clumping of cells. After PBS washing, the cells were resuspended in a 100 μl/well of permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) and incubated for 2 min on ice. Finally, K562 cells were processed by the "In situ cell death detection kit, Fluorescein"