Journal of Photochemistry & Photobiology, B: Biology 178 (2018) 505–511

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Modulation by neighboring cells of the responses and fate of melanoma cells irradiated with UVA

Aleksandra Krzywon, Maria Widel*, Krzysztof Fujarewicz, Magdalena Skonieczna, Joanna Rzeszowska-Wolny

Biosystems Group, Institute of Automatic Control, Faculty of Automatics, Electronics and Informatics, Silesian University of Technology, 16 Akademicka Street, 44-100 Gliwice, Poland

ARTICLE INFO

Keywords:
Ultraviolet radiation
Human malignant melanoma cells
Human fibroblasts
Apoptosis
Reactive oxygen and nitrogen species
Interleukins 6 and 8

ABSTRACT

UVA radiation, which accounts for about 95% of the solar spectrum, contributes to and may be the etiological factor of skin cancers of which malignant melanoma is the most aggressive. UVA causes oxidative stress in various types of cells in the skin, keratinocyte, melanocytes, and fibroblasts, which is responsible for its cytotoxic effect. Here we used a transwell system to explore how the responses of melanoma cells to a low dose of UVA (20 kJ/m^2, ~10% of the minimal erythema dose) are influenced by neighboring co-cultured melanoma cells or fibroblasts. This dose had a low toxicity for melanoma cells, but after irradiation, co-culture with non-irradiated melanoma cells caused a strong decline in their viability and an increased frequency of apoptosis, whereas co-culture with fibroblast exerted a protective effect on irradiated melanoma cells. At the same time, the presence of non-irradiated cells, especially fibroblasts, decreased the level of UVA-induced reactive oxygen and nitrogen species. Interleukins efficiently produced by fibroblasts seem to be main players in these effects. Our studies reveal that coexistence of fibroblasts with melanoma cells may strongly modulate the direct action and may change bystander effects exerted by UVA light. Similar modulation of the effect of UVA on melanoma cells *in vivo* by bystander-like signaling from neighboring cells would have consequences for the development of malignant melanoma.

1. Introduction

UV radiation (UVR) is a carcinogenic agent responsible for induction of skin cancers, basal and squamous cell carcinomas [1], and malignant melanoma, the most aggressive skin neoplasm [2–4] whose incidence is increasing worldwide in white population [5,6]. UVA, which constitutes about 95% of the solar spectrum reaching the earth, is potentially the most dangerous wavelength due to its atmosphere- and tissue- penetrating ability (1000 μm, reaching hypodermis) [7]. Exposure to UVA during artificial tanning may also play a role in melanoma development [8]. Cells in all three layers of dermal tissues, the epidermis containing mainly keratinocytes and melanocytes, the dermis containing fibroblasts, and the subcutaneous tissue can be chronically exposed to UVA in sunlight. UVA does not damage DNA directly but causes damages to DNA bases, proteins, and lipids by generating reactive oxygen species (ROS) [9] including singlet oxygen ($^1O_2$) and hydroxyl free radicals ($^{\cdot}O$), especially in the presence of oxygen and endogenous photosensitizers (porphyrins, heme- containing proteins). All three bands of UVR (UVA, UVB, and UVC) generate ROS and especially superoxide ($O_2^{\cdot -}$) in human dermal fibroblast, not only in the irradiated cells but also in bystander fibroblast co-incubated with them [10].

In the skin, melanocytes which may bear mutations predisposing to melanoma [11–14], but which are not yet recognized as such co-exist with other cell types, mainly fibroblasts. Here we have explored to what extent other adjacent cells modulate the response of melanoma cells to UVA radiation through bystander-like mechanisms. We used an experimental system, which reproduces to some extent the situation in skin in vivo, in which human melanoma cells were irradiated with UVA, co-incubated without or with non-irradiated cells of the same line or with human dermal fibroblast, and assayed for viability, apoptosis and senescence. These responses were compared with deregulation of oxidative equilibrium, impairment of mitochondrial membrane potential, and production of proinflamatory cytokines IL-6 and IL-8 as mediators of cellular communication.

* Corresponding author at: Institute of Automatic Control, 16 Akademicka Street, 44-100 Gliwice, Poland.
E-mail address: maria.widel@polsl.pl (M. Widel).

https://doi.org/10.1016/j.jphotobiol.2017.12.012
Received 11 October 2017; Received in revised form 7 December 2017; Accepted 8 December 2017
Available online 09 December 2017
1011-1344/

2. Materials and Methods

2.1. Cells Lines and Co-culture

Human malignant melanoma cells (line Me45) and neonatal human dermal fibroblast (NHDF—Neo, Lonza) were used. Human malignant melanoma Me45 cell line (derived from a lymph node metastasis of primary skin melanoma located on the temple in a 35-year-old male) was established in 1997 at the Radiobiology Department of the Centre of Oncology in Gliwice. Although primary melanoma removed surgically 12 month ago was pigmented, the metastatic cells showed only trace of pigmentation. A reason probably lies in the fact that exposure to sunlight induces pigmentation as a radiation defense which is not necessary for lymph nodes. Therefore identity of melanoma cells was tested and confirmed by immunocytochemical reaction with DAKO monoclonal antibodies HMB 50, S-100 and Melan A [15].

Both cells types were grown in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Ham medium (Sigma- Aldrich) with 12% fetal bovine serum (PAA), without antibiotics.

The co-culture system was essentially as described in detail in [10]. Briefly, about 20 h before experiments 1×10^5 Me45 cells were seeded into the wells of 6-well dishes (BD Falcon) and the same number of Me45 cells and NHDF fibroblast cells to be neighbors was seeded into corresponding inserts placed in separate 6-well dishes. After removing the medium from wells, the Me45 cells were irradiated (cover open) at room temperature with 20 kJ/m^2 of UVA ($\lambda = 365$ nm) generated by a UV crosslinker (CL-1000 model, UVP, Upland, CA, USA). Deprivation of the cells of the medium during the radiation session (6 min 48 s) was not stressful for cells as checked in pilot study for the control cells, also devoid of medium for the same time. Exposure to normal light during irradiation was limited by the UV lamp housing.

Immediately after irradiation, 2 ml of fresh medium was added to the wells and the irradiated cells were then cultured alone or co-cultured with non-irradiated cells growing in inserts.

Three experimental regimens were used:

1. Me45 cells were irradiated and incubated without inserts containing neighbors,
2. Me45 cells were irradiated and co-incubated with non-irradiated Me45 cells,
3. Me45 cells were irradiated and co-incubated with non-irradiated NHDF fibroblasts.

Before insertion of inserts into wells, medium was replaced by 2 ml of fresh one, thus the volume of medium for 10^5 cells was identical in all three groups. Cells were then incubated in standard conditions (5% CO_2, 80% humidity, and 37 °C) for the desired time. Non-irradiated Me45 cells and NHDF cells in the specified systems were considered as the relevant controls.

2.2. Viable Cells Assays

Cells were harvested by trypsinization separately from inserts and wells and viability was measured by MTS assays (Promega) [10]. Viabilities are expressed as percentage change from the initial value.

2.3. Assay of Apoptotic, Necrotic, and Senescent Cells

Apoptotic and necrotic cells were quantitated by flow cytometry after labelling using Anexin V/FITC/PI kits (Invitrogen) [10]. Necrotic cells were ~1% of the total in all samples and were therefore not considered further. Assays of senescent cells after fixing cells in situ in wells and inserts were based on β-galactosidase expression using Senescence Cells Histochemical Kit (Sigma-Aldrich) [10]. A single investigator experienced in microscopy (MW) performed analyses using an inverted microscope (Zeiss), evaluating ≥ 1000 cells in each well and insert.

2.4. Assay of ROS, Superoxide Radical Anions, and Nitric Oxide (NO)

Total ROS in cells were assayed using 2', 7'- dichlorofluorescein diacetate (DCFH-DA, Sigma–Aldrich) and superoxide ($O_2^{\cdot -}$) using MitoSOXRed mitochondrial superoxide indicator (Invitrogen), and quantitated by flow cytometry [10]. NO was assayed by the reagent 4-amino-5-methylamino-2', 7'-dichlorofluorescein diacetate (DAF-FM diacetate, Invitrogen) [10]. Data expressing the percentage increase of ROS, superoxide and NO levels with respect to adequate controls are presented as means ± SD calculated on the base of three independent experiments.

2.5. Mitochondrial Membrane Potential

Mitochondrial transmembrane potential ($\Delta\psi_{mit}$) was assayed using tetramethylrhodamine ethyl ester (Sigma–Aldrich) and quantitated by flow cytometry [10]. Results from three experiments are presented as means of change of fluorescence intensity relative to relevant controls.

2.6. Assay of Interleukins

The levels of interleukin 6 (IL-6) and interleukin 8 (IL-8) were determined in culture media shared by co-incubated cells or from media of irradiated cells incubated alone, using the quantitative sandwich enzyme-linked immunosorbent assay (ELISA, immunologic kits of R&D Systems). The procedures were consistent with protocols described in [10].

2.7. Statistical Analyses

All data are presented as means ± SD from three independent experiments. Comparisons between groups and significances levels were calculated by Students' *t*-test and *p*-value ≤ 0.05 between groups were considered as significant.

3. Results

3.1. Neighboring Cells Influence the Viability of Melanoma Cells

Melanoma cells growing in the wells of a co-cultured system were separated by a membrane with 0.4 μm pores from melanoma cells or fibroblasts growing in inserts, allowing free diffusion of molecules between the media surrounding the two cell types. The viability of non-irradiated melanoma cells was increased somewhat when they were co-cultured with fibroblasts (Fig. 1A) but the growth of irradiated melanoma cells was not significantly affected (Fig. 1B). Unexpectedly, the viability of irradiated melanoma cells was reduced markedly when they were co-cultured with non-irradiated melanoma cells (Fig. 1B).

3.2. The Frequency of Apoptosis in Irradiated Melanoma Cells Is Reduced by Co-culture with Fibroblast

The frequency of apoptosis in control melanoma cells varied between 1 and 1.5% (data not presented. The frequency of apoptotic melanoma cells increased significantly in all irradiated groups when compared with corresponding controls. Co-culture of irradiated melanoma cells with non-irradiated cells of the same line resulted in significant increase of apoptosis, whereas co-culture with non-irradiated fibroblasts reduced it markedly (Fig. 2).

3.3. Senescence Is Induced Only in Irradiated Melanoma Cells Incubated without Bystander Cells

Premature senescence in control melanoma cells was negligible and