

INDUCTION OF BYSTANDER EFFECT IN HUMAN MALIGNANT MELANOMA CELLS BY DIFFERENT SPECTRUM OF ULTRAVIOLET RADIATION

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Abstract

Bystander effect is the phenomenon where molecular signals produced by directly irradiated cells cause different biological changes in unirradiated neighbors. The knowledge about the bystander effect after UV radiation is very limited. UV light covers three ranges: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). The mechanism of action of these three wavelength ranges varies and also different type of cells may respond differently. Furthermore, one can expect a mutual signaling between exposed and non exposed cells. In this study I want to answer the question whether the neighborhood NHDF fibroblasts influence the survival and apoptosis in Me45 melanoma cells after exposure to different UV spectrum and if the bystander effect is present at all.

1. Introduction

The bystander effect can be defined as communication between directly irradiated and neighboring non- irradiated cells. Signals from irradiated cells produce different responses in bystander cells which result in cell death, chromosomal abnormality, sister chromatin exchange, micronuclei formation and DNA damage. Ionizing and ultraviolet radiation exhibit bystander effect, but the knowledge about UV radiation induced bystander effect is limited. UV is divided into three bands: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm)[1, 2]. Because the ozone layer completely absorbs UVC and shorter UVB (below 300 nm), UVA is a predominant part of sunlight that reaches the Earth (~95%) [3]. The rest of the spectrum of UVB rays reaching the Earth's surface is ~5% [4,5]. UVA and UVB rays act differently on cells. UVA reacts in the presence of oxygen producing reactive oxygen species, which can indirectly damage DNA. In contrast, UVB produces specific DNA damage such as cyclobutane pyrimidine dimers (CPDs), pyrimidine 6-4 pyrimidone photoproducts (6-4PPs). Generally, UV light causes oxidative stress in directly hit cells

through the production of reactive oxygen species [6].

2. Material and methods

2.1 Cell line and experimental protocol

Human malignant melanoma (Me45) cell line (derived from a lymph node metastasis of skin melanoma in a 35-year-old male) and normal human dermal fibroblast (NHDF) were obtained from the bank of the Center of Oncology in Gliwice. Cells were grown as monolayer cultures in Dulbecco's modified Eagle's DMEM/F12 HAM medium (Sigma), supplemented with 12% of fetal bovine serum (PAA, Immuniq) and 80µg/ml gentamicin (Krka), and were incubated at 37°C in humidified air containing 5% CO₂. About 20 h before irradiation cells were seeded (100 000 cells/well in 2 ml medium) into 6-well dishes. The same number of cells were seeded on inserts, the cells not designed to be irradiated (bystander cells). The permeable bottom membrane of the insert allows the diffusion of the medium components through 0.4 µm pore size, but separate the direct contact of both type of cells. Just after irradiation medium was removed and cells in wells were irradiated with different doses of UVA (20 KJ/m²), UVB (10 KJ/m²) and UVC (200 J/m²), generated by proper UV Crosslinkers. After irradiation 2ml fresh medium was added and inserts with non irradiated cells were put in into wells. Cells were co- cultured for required time before proper tests.

2.2 Proliferative activity (MTS) assay

MTS-tetrazolium reduction assay (The Cell Titer 96® AQueous One Solution Cell Proliferation Assay, Promega) was used for determining the number of viable cells. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt) is reduced by viable cells into intensely colored formazan. The more viable cells in culture conditions, the larger amount of formazan color is produced. Irradiated cells were co-incubated with non-irradiated for 24, 48 and 72h. In the proper time cells from wells and insert were harvested

separately and mixed with MTS reagent (according to manufacture protocol). Suspension was transferred to 96- well plate and incubated for 60 min at 37°C in a humidified, 5% CO₂ atmosphere. The absorbance was recorded at 490 nm using universal plate reader.

2.3 Apoptosis assay

The Dead Cell Apoptosis Kit with Annexin V-FITC (Invitrogen) was used for detection of cell apoptosis. In proper time cells from wells and inserts were harvested, spun down, washed with PBS, suspended in annexin V- staining buffer, and incubated for 15 min with Annexin V-FITC per the manufacturer’s instructions. Cells were analyzed in BD FACS flow cytometer (Becton Dickinson) using the fluorescence excitation/emission maxima: FITC annexin V: 494/518 nm.

3. Results and discussion

In this article I want to answer the question whether the neighborhood NHDF decrease survival and apoptosis in Me45 after UV radiation. Since energy of UV increase with decreasing wavelength, I applied following doses: UVA: 20 kJ/m², UVB: 10 kJ/m² and UVC: 200 J/m².

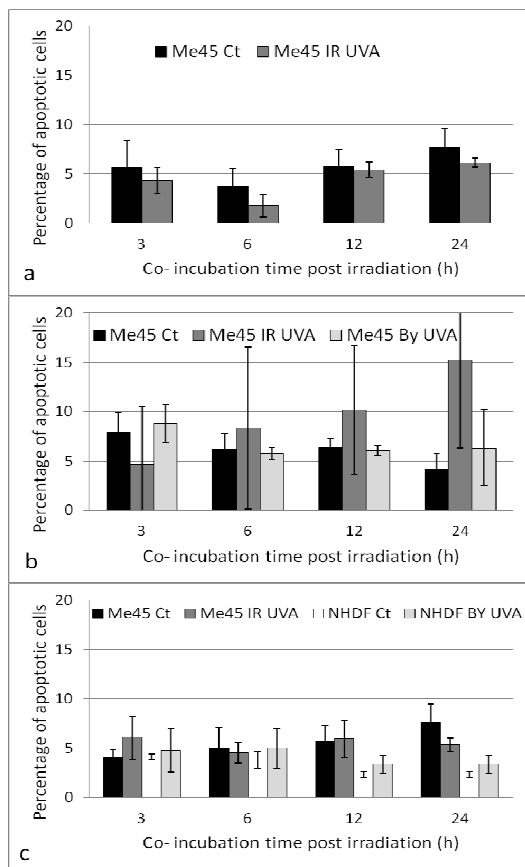


Fig.1. Frequency of apoptosis in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVA radiation.

Analysis of apoptosis indicates that UVA radiation did not induce apoptosis in irradiated Me45 cells incubated without neighbor cells (Fig. 1a). However, when irradiated cells were co-incubated with non-irradiated Me45 neighborhood, apoptosis in directly irradiated cells reach ~3-fold of control level in 24 hours wherein non irradiated Me45 cells do not exhibit apoptotic death (Fig. 1b). Fig. 1c show that non-irradiated NHDF cells decreased apoptotic frequency in directly irradiated Me45 cells (Fig. 1a vs. Fig. 1b and Fig 1c).

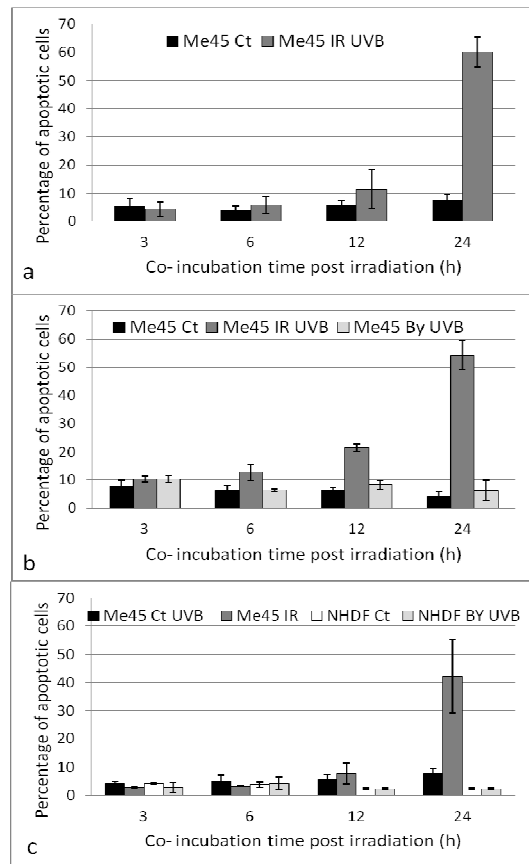


Fig.2. Frequency of apoptosis in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVB radiation.

Irradiated Me45 cells incubated alone showed that frequency of apoptotic cells increases in time after UVB radiation reaching the 6 fold over control at 24 h (Fig. 2a). Co-incubation with non- irradiated Me45 cells does not change apoptosis in irradiated Me45 cells in comparison with that incubated alone (Fig. 2a vs. Fig 2b). However, irradiated Me45 cells co- incubated with non- irradiated NHDF cells show decrease of apoptosis indicating a protective effect caused by fibroblasts (Fig. 2c).

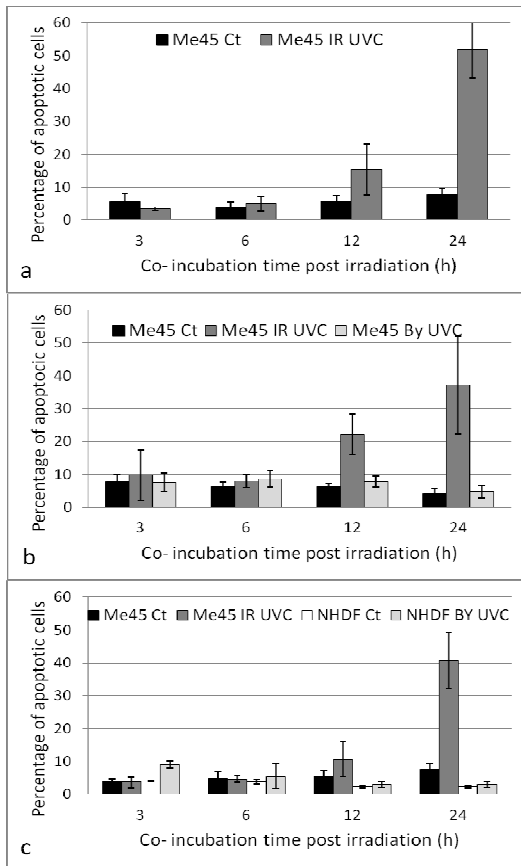


Fig.3. Frequency of apoptosis in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVC radiation.

Fig. 3a shows that frequency of apoptosis in UVC irradiated Me45 cells incubated alone reaches ~5 fold over control in 24 hours. The similar curve of apoptosis was observed for irradiated Me45 cells co-incubated with non-irradiated Me45 cells. (Fig. 3a vs. Fig. 3b). Co-incubation of irradiated Me45 cells with non-irradiated NHDF cells does not show protective effect of co-culture (Fig. 3c).

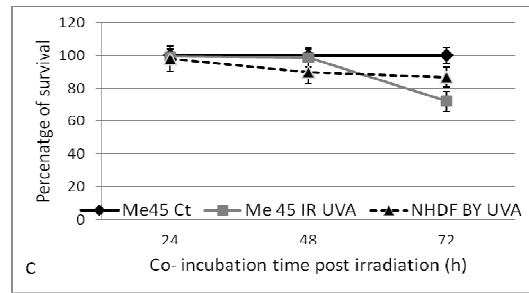
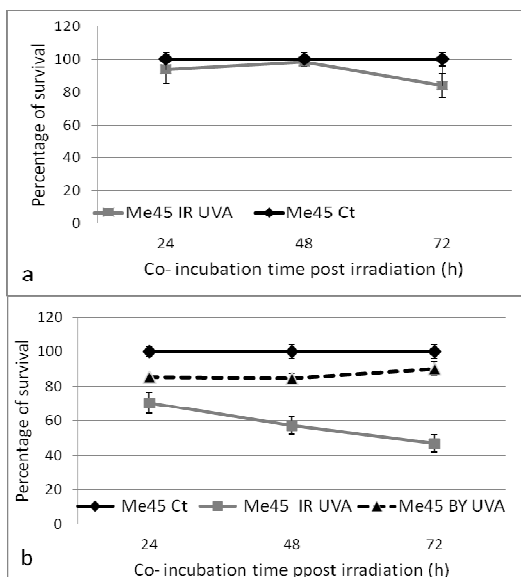


Fig.4. Proliferative activity in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVB radiation.

UVA exposed Me45 cells incubated alone show a slight decrease of survival in time, reaching ~80% of control at 72 hours (Fig. 4a). In experiment where irradiated Me45 cells were co-incubated with neighbors of the same line, at 72 h viability of directly irradiated cells was ~45% and in non-irradiated cells it is reduced only to about 80% (Fig. 4b). Co-incubation of irradiated Me45 cells with non-irradiated NHDF appears to increase survival of directly irradiated Me45 cells to ~65% (Fig. 4b vs. Fig. 4c). The viability of bystander fibroblasts is on the level ~80% (Fig. 4c).

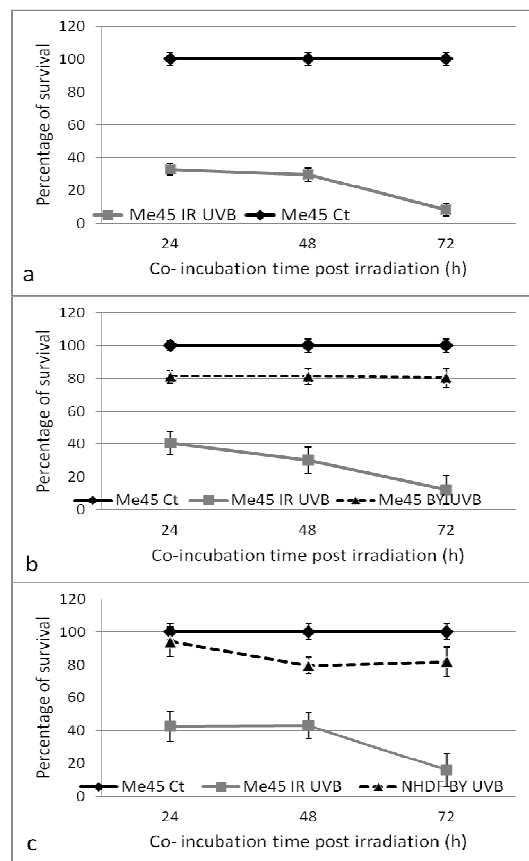


Fig.5. Proliferative activity in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVB radiation.

Results indicate that exposition of Me45 cells to UVB radiation without co-incubation shows large decrease in survival (~10% living cells after 72 hours post irradiation) (Fig. 5a). Similar survival curves are

observed for Me45 exposed to UVB regardless co-incubation is performed with non-irradiated Me45 or non-irradiated NHDF (Fig. 5b vs. Fig. 5c). Bystander NHDF fibroblasts do not show protective effect on UVB irradiated melanoma cells (Fig. 5c).

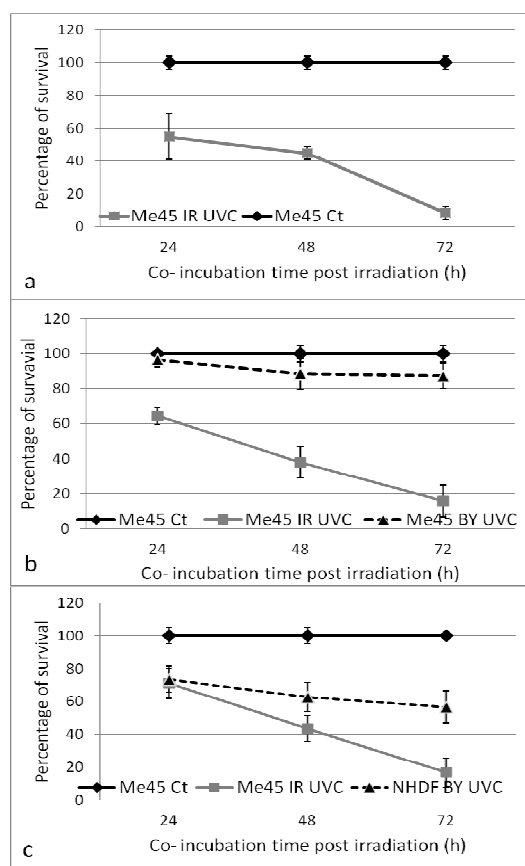


Fig.6. Proliferative activity in control (Ct) and bystander (BY) Me45 and NHDF cells co-cultured with Me45 cells (IR) exposed to UVC radiation.

UVC radiation causes significant decrease of survival in directly irradiated Me45 cells. There was no difference whether the Me45 was incubated alone or co-incubated with the same line or co-incubated with NHDF. In all cases at 72 h we had only ~10% living cells in directly irradiated Me45 (Fig. 6). However, UVC radiation significantly decreases survival of bystander NHDF cells of ~20% in comparison to UVA and UVB radiation (Fig. 6c vs. Fig. 4c and Fig 5c).

In this study I observed a protective effect of non irradiated NHDF on UVA- irradiated Me45, manifested as significant diminution of frequency of apoptotic cells in irradiated Me45 cells. This result are in agreement with Widel et al. [7] who showed that bystander NHDF reduce frequency of apoptotic cells in X-ray irradiated Me45 cells (2Gy and 4Gy). Protective effect was not observed when melanomas was irradiated by UVB or UVC rays. In the case of apoptotic cells I did not see bystander effect in non-irradiated cells co-incubated with irradiated Me45 cell after UVA, UVB and UVC exposition. Bystander effect measured by MTS assay in Me45

melanoma cells was generally weak for all three bands of UV radiation. UVA and UVB also induced only weak bystander effect in NHDF cells. However UVC at dose 200/m² was very effective in generation of bystander effect in NHDF fibroblasts as measured by decrease of survival.

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