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Bystander Effect Induced by UV Radiation; why should we be interested?*

Efekt sąsiedztwa indukowany promieniowaniem UV; dlaczego powinniśmy się zainteresować?

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Summary

The bystander effect, whose essence is an interaction of cells directly subjected to radiation with adjacent non-subjected cells, via molecular signals, is an important component of ionizing radiation action. However, knowledge of the bystander effect in the case of ultraviolet (UV) radiation is quite limited. Reactive oxygen and nitrogen species generated by UV in exposed cells induce bystander effects in non-exposed cells, such as reduction in clonogenic cell survival and delayed cell death, oxidative DNA damage and gene mutations, induction of micronuclei, lipid peroxidation and apoptosis. Although the bystander effect after UV radiation has been recognized in cell culture systems, its occurrence *in vivo* has not been studied. However, solar UV radiation, which is the main source of UV in the environment, may induce in human dermal tissue an inflammatory response and immune suppression, events which can be considered as bystander effects of UV radiation. The oxidative damage to DNA, genomic instability and the inflammatory response may lead to carcinogenesis. UV radiation is considered one of the important etiologic factors for skin cancers, basal- and squamous-cell carcinomas and malignant melanoma. Based on the mechanisms of actions it seems that the UV-induced bystander effect can have some impact on skin damage (carcinogenesis?), and probably on cells of other tissues. The paper reviews the existing data about the UV-induced bystander effect and discusses a possible implication of this phenomenon for health risk.

Key words: ultraviolet radiation • bystander effect • genomic instability • oxidative stress • skin cancers • malignant melanoma

Streszczenie

Popromienny efekt sąsiedztwa (bystander effect), którego istotą jest interakcja komórek ekspozowanych na promieniowanie z sąsiadującymi komórkami nienapromieniowanymi, za pomocą sygnałów molekularnych, jest ważnym elementem działania promieniowania jonizującego. Jednak znajomość efektu sąsiedztwa w przypadku promieniowania ultrafioletowego (UV) jest dość ograniczona. Reaktywne formy tlenu i azotu generowane w komórkach ekspozowanych na promieniowanie UV indukują efekty sąsiedztwa w komórkach nieekspozowanych na UV, ujawniające się w postaci: obniżenia przeżywalności komórek klonogennych i opóźnionej śmierci komórkowej, oksydacyjnych uszkodzeń DNA i niestabilności genetycznej, indukcji mikrojąder, peroksydacji lipidów i apoptozy. Chociaż efekt sąsiedztwa po ekspozycji na promieniowanie UV został zaobserwowany w systemach komórkowych *in vitro*, jego występowanie w warunkach *in vivo* nie było

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badane. Jednak promieniowanie słoneczne, które jest głównym źródłem promieniowania UV w środowisku, indukuje w ludzkiej skórze stany zapalne i immunosupresję, procesy, które mogą być uważane za efekty sąsiedztwa wywołane promieniowaniem UV. Oksydacyjne uszkodzenia DNA, niestabilność genetyczna i reakcje zapalne mogą prowadzić do kancerogenezy. Promieniowanie UV jest uważane za jeden z ważnych czynników etiologicznych nowotworów skóry, tj. raka podstawno-komórkowego i płaskonabłonkowego oraz czerniaka złośliwego. Na podstawie mechanizmów działania wydaje się, że efekt sąsiedztwa wywołany promieniowaniem UV może mieć pewien udział w uszkodzaniu (kancerogenezie?) skóry i prawdopodobnie komórek w innych tkankach. Celem artykułu jest przegląd istniejących danych na temat indukowanego przez UV efektu sąsiedztwa oraz zwrócenie uwagi na możliwe implikacje tego zjawiska dla ryzyka zdrowotnego.

Słowa kluczowe: promieniowanie ultrafioletowe • efekt sąsiedztwa • niestabilność genetyczna • stres oksydacyjny • nowotwory skóry • czerniak złośliwy

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INTRODUCTION

It has been repeatedly demonstrated that ionizing radiation induces bystander responses in cells and tissues [58,59,61,71,79,98,100]. However, ionizing radiation is not unique in generating these phenomena. A few reports suggest that bystander responses are also generated by other stressors, including chemotherapeutic drugs [1], ultraviolet light [5,16,19,20,21] and photodynamic treatment [12,15,18]. UV radiation is a known carcinogenic agent responsible for induction of skin cancers, basal and squamous cell carcinomas [23] and malignant melanoma [66,74,85]. Malignant melanoma is a highly aggressive cancer whose morbidity is constantly increasing [101]. The highest rate of melanoma morbidity is recorded in Queensland, Australia (about 40–60 cases per 100 thousand inhabitants per year) and in the USA (10–20 cases per 100 thousand). Although Poland is a country with low incidence of melanoma (2–5 per 100 thousand inhabitants) its growth by 2.6% per year among men and 4.4% among women is also recorded [47]. The risk of melanoma rises with increasing exposure to UV radiation [6], especially in the population with very fair skin, prone to sunburn, and exposed to sunlight in childhood. Furthermore, widely used artificial UV for indoor tanning, particularly by young people, contributes to epidemiologically proven skin cancer, mainly melanoma [25,92].

UV radiation covers the three solar spectrum ranges: UVA, 320–400 nm (long-wave), UVB 290–320 nm (middle-wave) and UVC, 200–290 nm (short-wave) [7,64]. The UVA band constitutes ~95% of the solar radiation, and UVB (above 300 nm) ~5% of the total solar UV radiation that reaches the Earth. The shorter UVB and UVC are almost completely absorbed by the protective ozone of the stratospheric layer [35], with exceptions where this layer is insufficient, e.g. areas over Australia, where destruction of atmospheric ozone has created the “ozone hole”.

The mechanisms of action of the three bands of UV radiation differ [73]. The short UV radiation, UVC, can penetrate the skin to a depth of approximately 60–80 micrometers. Radiation below 300 nm is particularly dangerous for cells and organisms because within this band are the absorption spectra of DNA and RNA (260 nm) and proteins (280 nm). UVB radiation can penetrate the skin to a depth of approximately 160–180 micrometers. UVB photons are thus absorbed primarily by keratinocytes and induce specific DNA damage: the cyclobutane pyrimidine dimers (CPDs; about 60–70%) and 6,4 photoproducts (PPs; 30–40%). These types of DNA damage lead to transition mutations C→T or CC→TT [77], and their accumulation, if not repaired, may lead to neoplastic transformation. These transitions are accepted as premutagenic lesions for UVB. The most frequent site of occurrence of these mutations is the p53 tumor suppressor gene [22,57]. CPDs and 6–4 PPs may cause primary as well as secondary DNA strand breaks [8,10,26,83,84]. However, UVA is considered potentially the most dangerous for health risk due to its atmospheric and tissue penetration properties [reviewed in 55]. UVA is especially active in the presence of oxygen and endogenous photosensitizers (porphyrins, heme-containing proteins) by generating reactive oxygen species [2]. The exposure to UVA induces a generation of singlet oxygen and hydroxyl free radicals, which can damage cellular macromolecules, such as proteins, lipids and DNA. However, oxidative stress is also induced by UVB, particularly in the form of hydrogen peroxide and lipid peroxidation [54,60]. Oxidative stress is an important mediator of bystander effects induced by ionizing radiation [33,100]. It is thus reasonable to expect that oxidative stress induced by UV radiation will also promote damaging effects in bystander cells, not exposed to UV. Published data indicate that UV-exposed cells secrete signaling molecules that generate in neighboring, non-exposed cells bystander responses such as different types of DNA damage,

Table 1. Summary of experimental data on bystander effect and genomic instability (GI) induced by UV radiation

Experimental system	Effect(s) determined	Mediator of bystander effect/GI	Publication
Human keratinocytes HaCaT cell line UVA	Persistent genomic instability: increase of micronuclei, decline of plating efficiency, increase of HPRT mutation	Hydrogen peroxide (catalase reduced the effects)	[70]
Chinese hamster fibroblasts V79 UVA, UVB	Reduced clonogenic survival, delayed mutation of HPRT gene, chromosomal damage	Oxidative stress for UVA	[20]
Human keratinocytes, HaCaT cell line UVA, UVB	Apoptosis	Nerve growth factor (NGF)	[5]
Chinese hamster fibroblasts V79 UVA, UVB	Genomic instability; delayed mutation	ROS (GSH, SOD, catalase reduced effect)	[21]
Human keratinocytes, HaCaT cell line; UVA	Reduction of clonogenic survival	ROS (DPI* reduced effect)	[55]
Murine malignant melanoma B16 cells; UVA, UVB	Induction of melanogenesis	ROS; influx of calcium ions	[65]

*DPI – diphenyleiiodonium, an inhibitor of NADPH oxidase.

including delayed mutations [19,20,21], micronucleus formation [70], diminution of survival [55,67] and apoptosis [5]. However, our understanding of the UV-induced bystander effect in unexposed cells is still very poor, even *in vitro*, and this phenomenon at the level of tissue and organisms is completely unknown. Thus, there is a great need to understand better the basic mechanisms of skin carcinogenesis and the possible participation of the bystander effect phenomenon in this process and its influence on other tissues.

UV-INDUCED REACTIVE OXYGEN AND NITROGEN SPECIES AS MEDIATORS OF BYSTANDER EFFECTS

Upregulation of oxidative metabolism in cells exposed to ionizing radiation and bystander cells suggests that biological effects in bystander cells may be a consequence of oxidative stress [4,91]. Used in some experiments, a radical scavenger, DMSO [33] or vitamin C [33,42] diminished the level of DNA damage in irradiated and bystander cells. This seems to confirm the role of reactive oxygen species (ROS) as initial signaling molecules. It is thus reasonable to expect that ROS generated by UV radiation in exposed cells may in turn induce a bystander effect in neighboring cells. Actually, the available data on UV-induced bystander effects and related genomic instability (GI) indicate ROS as signaling molecules in the phenomena (Table 1).

The biological effect of UV radiation is dependent on its spectral distribution. Among the three ranges of solar UV radiation, UVB is known to be highly detrimental to cells since it may directly affect nucleic acids and change many biological processes. Additionally, UVB exposure can lead to oxidative modifications of proteins and lipids in membrane structures, which are the initial steps in photoaging and photocarcinogenesis [63]. Oxidative stress induced by UVB appears particularly in the form of hydrogen peroxide and lipid peroxidation [54,60], although hydrogen

peroxide can also be generated by UVA [70]. An increase of lipid peroxidation, changes in antioxidant enzyme activities, apoptosis, and formation of sunburn cells in cultures have been reported after UVB radiation [80], while exposure to UVA mainly acts through the generation of reactive oxygen species such as singlet oxygen and hydroxyl free radicals, which can also damage cellular macromolecules: DNA, proteins and lipids. The oxidative stress induced by UVA radiation has been shown to cause increased 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) lesions, which result from the oxidation of deoxyguanosine moieties [78]. Oxidized nucleosides and bases are potentially mutagenic and are important in inducing genomic instability and carcinogenesis [48,49,76]. In the case of ionizing radiation endogenously generated ROS play a key role in the induction of DNA damage in bystander cells appearing as chromosomal aberrations, micronuclei, sister chromatid exchanges, mutations, genome instability, and neoplastic alterations [reviewed in 98]. Furthermore, it has been shown that oxidatively damaged DNA which arises as a result of locally applied radiotherapy may be involved in the normal tissue side effects [75]. One can thus expect that UV-induced ROS will lead to damaging consequences also in tissues not subjected to radiation. However, to date data on UV-induced bystander effects *in vivo* are not available. But, it has been shown that UVA-irradiated HaCaT human skin keratinocytes induced a bystander effect in unirradiated cells co-incubated with them [55]. This effect was demonstrated as a reduction of clonogenic cell survival. Pretreatment of bystander cells with diphenyleiiodonium (DPI), which is an inhibitor of NADPH oxidase, reduced the induction of the bystander effect, indicating that this enzyme, participating in ROS induction, plays a role in bystander signaling.

UV-induced oxidative stress leads to lipid peroxidation. Malondialdehyde (MDA), 4-hydroxynonenal (4HNE) and other lipid peroxidation byproducts are small molecules which are able to diffuse within and between cells

and can form adducts with proteins, DNA and phospholipids in bystander cells. Created DNA adducts are potentially mutagenic and carcinogenic [53,102]. However, the end-products of lipid peroxidation also have properties of secondary messengers, that can either activate the signal cascade leading to repair of DNA damage and its stabilization, or to apoptosis [30]. Lipid peroxidation products generated in UV-exposed dermal tissue may be signaling molecules inducing bystander effects in surrounding tissues, or in circulating cells, most likely with damaging consequences. This supposition is confirmed by elevated MDA levels in normal skin biopsies surrounding superficial melanoma characterized by high levels of ROS [81].

It is necessary to mention that UVA radiation, which comprises 95% of the solar ultraviolet radiation reaching the earth's surface, shows an inverse dose rate effect [87]. Similar inverse dose rate effects are sometimes observed after ionizing radiation [72,94]. Following UVA irradiation with equivalent doses at different dose rates (22–80 J/m²/s), HaCaT cells developed after lower dose rates more micronuclei, more DNA damage measured with comet assay, and especially a high level of persistent genomic instability and high level of lipid peroxidation (MDA/4-HNE), whose role in bystander effects is mentioned above. These effects were reversed using catalase, indicating a role for hydrogen peroxide in this phenomenon [87].

Both spectrums of solar radiation, UVB and UVA, can cause immunosuppression [11,32,43]. Immune suppression, which is in fact a bystander effect of UV radiation, may be a risk factor for skin cancer susceptibility. Bystander effects and genomic instability reflect an inflammatory-type response, a feature of which is production of cytokines and free radicals [50]. One of the effects of UV-induced inflammatory-type response may be the generation by epidermal cells of inflammatory mediators which can promote carcinogenesis and enhance tumor growth [89]. UV radiation at sufficiently high doses induced inflammation in the skin; however, sub-inflammatory doses were sufficient to induce mutagenesis [32]. UV intensifies blood flow and infiltration from the blood of such cells as macrophages and neutrophils into the skin, resulting in clinically observed inflammation and increased production of nitric oxide (NO) and prostaglandins, which contribute to this process [31]. NO plays an important role in many biological processes, often opposite, such as stimulation of proliferation or apoptosis, mainly depending on the concentration [86]. UV radiation-induced lipid peroxidation increases production of prostaglandins, including PGE₂, which in turn causes inflammation in dermal tissue. Animal experiments indicate that UV-exposed keratinocytes activate a cytokine cascade including PGE₂ > IL-4 > IL-10 that ultimately results in systemic immune suppression [88]. UV radiation also activates nitric oxide synthase (iNOS), which generates NO that participates in immune suppression by induction of apoptosis and loss of dendritic cells from the epidermis [44]. Thus, NO induced by UV radiation can also lead to variable effects in non-exposed cells, not necessarily residing in dermal tissue.

CELL SURVIVAL, DNA DOUBLE STRAND BREAKS AND MICRONUCLEI INDUCED IN UV-EXPOSED AND BYSTANDER CELLS

UVA radiation does not induce DNA damage directly, because it is not absorbed by native DNA. The biological effects of UVA depend on the presence of oxygen and endogenous photosensitizers. The UVA induces oxidative stress in exposed cells [2,70], which are a mediator of the bystander effect. It has been shown in a co-incubation system that UVA at a dose of 100 kJ/m² induced a bystander effect in human HaCaT keratinocytes and MRC5 fibroblasts manifested as reduced clonogenic cell survival, whereas much more energetic UVB did not, even at a dose of 400 J/m² [97]. The bystander effect was induced between cells of the same line and between two cell lines. The diminution of cell survival in UVA exposed HaCaT keratinocytes and non-exposed HaCaT keratinocytes co-incubated with them has also been shown in another paper of the same research group [55], where inhibition of ROS generation also inhibited the bystander effect. Whereas UVA acts through generation of oxidative stress, UVB and UVC radiation can damage DNA directly, inducing the cyclobutane pyrimidine dimers and 6,4 photoproducts. These types of DNA damage may cause mutations and primary, as well as secondary, DNA strand breaks [8,10,26,83,84]. The bystander effect in UVB exposed cells is mainly shown as delayed mutations and genomic instability, and these aspects are described in the next section.

Induction of micronuclei is a marker of DNA damage and genomic instability in directly irradiated and bystander cells [98,100]. The DNA-damaging potential of UVB and UVA radiation was investigated by analyzing the frequency of micronuclei (MN) in human melanocytes and fibroblasts [28]. Cultured melanocytes and fibroblasts were exposed to physiological doses of ultraviolet A (150 kJ/m² equivalent to 0.2 minimal erythema dose) or ultraviolet B (10 kJ/m² equivalent to 1 minimal erythema dose) and, for comparison, to 1 Gy of gamma rays. The results indicate that UV doses can induce chromosomal damage in a yield comparable with that observed after exposure to approximately 1 Gy of ionizing radiation. A dose of 1 Gy is sufficient to effectively induce micronuclei in bystander cells [e.g. 39]. Although the bystander effect was not studied in the presented experiment [28], it is very probable that physiological doses of UVA and UVB can induce micronuclei also in bystander cells.

There is a lack of literature data on the bystander effect induced by UVC radiation. Using a transwell co-incubation system (wells with inserts) which allows the diffusion of medium components through the permeable membrane bottom of the insert (0.4 μm pore size), but does not allow the direct contact of both types of cells, we observed that K562 leukemia cells irradiated with UVC (10–500 J/m²) revealed micronuclei in exposed cells and in bystander counterparts. Interestingly, the frequency of cells with micronuclei and the number of micronuclei per binucleated cell were even higher for bystander than for directly exposed cells (Fig. 1, A. Biskup¹). However, the mechanism of this effect needs to be elucidated. One reason for the higher yield of micronuclei in bystander cells may be the differences in

¹ A. Biskup, Radiation induced bystander effect in lymphoid cancer cells. Engineering diploma in biotechnology, Silesian Technical University, Gliwice, 2011.

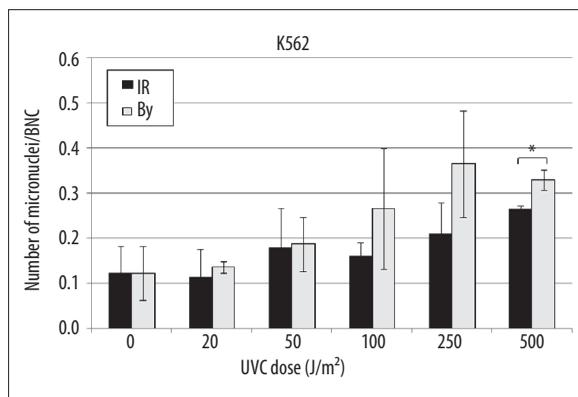


Fig. 1. Number of micronuclei per cell in binucleate K562 leukemia cells irradiated with UVC (dose range 10–500 J/m²) and in bystander cells co-incubated with them (* $p < 0.05$, Student's *t*-test)

proliferation rate of cells directly exposed to UVC and of bystander cells as expressed by the nuclear division index (NDI) [27], which decreased by ~30% in exposed cells but was practically unchanged in bystanders (not presented).

Studies performed also in our laboratory (A. Krzywon²) on HCT116 colorectal carcinoma cells exposed to 50 J/m² UVC (250 nm) indicated that γ H2AX foci appeared shortly after irradiation in exposed cells, but were slightly shifted in time in non-exposed neighbors co-incubated with them in inserts (Fig. 2).

Since CPDs and 6,4 PP are not formed in bystander cells, thus DSB visualized as γ H2AX foci must be generated by some molecular signals transmitted via medium by UV-exposed cells. The biochemical nature of these signals needs to be clarified.

UV-INDUCED GENOMIC INSTABILITY

The bystander effect and genomic instability seem to be related. The term “genomic instability” means the destabilization of the genome, observed as delayed reproductive death (DRD) in distant cell generations, the lethal mutations, chromosomal instability and aberrations transmitted to future generations of cells previously exposed to radiation [36,38,52,56], also known as “vertical transmission” [62]. Radiation induced genomic instability can probably contribute to tumor and normal tissue response to radiotherapy treatment and may lead to secondary tumors after radiotherapy [34]. Genomic instability is also observed after UV radiation. The mechanism of UVA-induced genomic instability differs from that of UVB radiation. Short-wave UVB induces mutations mainly through formation of pyrimidine dimers, whereas other types of DNA damage, such as oxidative base damage, are thought to be the premutagenic lesions for long-wave UVA light. However, formation of pyrimidine dimers, being premutagenic lesions, can also be generated by UVA. Furthermore, the C→T transitions as well as CC→TT tandem mutations occurred with both UVA and UVB light [69,78].

It was found that UVA induced persistent genomic instability in immortalized human skin keratinocytes, the HaCaT

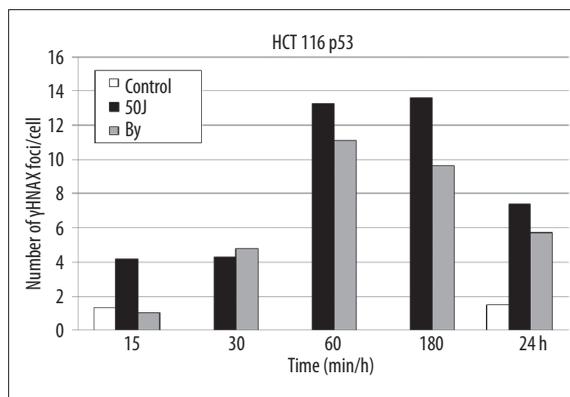


Fig. 2. Number of phosphorylated histone H2AX foci per cell in HCT116 colorectal carcinoma irradiated with 50 J/m² UVC and bystander cells co-incubated with them

cell line treated with a dose of 100 kJ/m², which equates to 30 min exposure in the midday sun during summer at latitude 48° north [70]. This instability appeared as delayed cell death manifested by decreased plating efficiency, increase of micronucleus frequency and hypoxanthine-guanine phosphoribosyl transferase (*hprt*) mutation when measured between 7 and 28 days following irradiation. UVA-irradiated cells demonstrated an increase in number of micronuclei compared with controls, reaching a 2.5-fold increase at day 7, and about 4-fold increase at days 14, 21, and 28 in the treated population. These populations represented approximately 7, 15, and 23 divisions at days 7, 14, and 21, respectively. Also *hprt* mutation increased about twofold in comparison with control at 7 days post irradiation [70]. The ability of UVA to induce genomic instability can, in consequence, lead to carcinogenesis in humans. Persistent genomic instability after UVA irradiation was also reported by O'Reilly and Mothersill [67], who found that human immortal keratinocytes had a prolonged reduction in plating efficiency (delayed cell death) after UVA and UVB, but no delayed cell death was expressed in the EPC fish cell line. It was also observed that delayed mutations occurred in the 10–20 generations of daughter V79 cells whose progenitors were exposed to UV (A and B) radiation [19,20,21]. Application of antioxidants such as GSH, SOD and catalase significantly reduced delayed mutation, especially in UVB exposed cells, indicating the participation of ROS in the process. Since external GSH caused an increase of the internal level of GSH, while SOD and catalase were not taken up by cells, these delayed mutations are likely to be a consequence of bystander effect signaling originating from irradiated cells and transmitted via medium [21]. UVA radiation produced a low level of immediate mutations in the *hprt* locus in V79 fibroblasts but a higher level of delayed mutations than UVB, or even X-ray radiation in clones derived from single cells which survived radiation. Furthermore, UVB induced about 5% of centrosome aberrations, whereas UVA did not induce centrosome aberrations at all, but increased variation in chromosome number [19,21]. This indicates that mechanisms leading to genomic instability differ for both UV spectra. However, short-term and delayed mutagenic events in critical genes as well as numerical chromosome aberration may be the first steps in carcinogenesis.

² A. Krzywon, Bystander effect induced by UV radiation in normal and cancer cells. Master thesis in biotechnology, Silesian Technical University, Gliwice, 2011.

APOPTOSIS INDUCED IN UV-EXPOSED AND BYSTANDER CELLS

Apoptosis plays an essential role in survival of organisms by preventing the proliferation of cells with mutated DNA, normal embryonic development, removal of damaged cells and maintenance of cell homeostasis. UV-induced cell death by apoptosis is considered a natural way for protection and a mechanism that eliminates damaged keratinocytes and bypasses the risk of malignant transformation [3]. Apoptosis in skin keratinocytes is manifested as sunburn cells and terminal differentiation. It has been found in *in vitro* conditions that HaCaT keratinocytes exposed to UV (A+B) developed apoptosis, which was visualized by MTT assay and DNA laddering [5]. The expression of Fas and Bax genes engaged in apoptosis regulation was also observed. Conditioned medium collected from UV exposed cells after 12 h induced bystander effects in non-exposed keratinocytes, which also elicited apoptosis, as shown by MTT assay, DNA electrophoresis and expression of pro-apoptotic genes Bax and Fas. It is interesting that high dilution of conditioned medium did not induce cell death but even stimulated proliferation of HaCaT cells. The authors hypothesize that conditioned media contain different soluble factors that operate through binding to different receptors for death or survival [5]. One of them can be NGF (nerve growth factor) secreted by keratinocytes that possess on the cell surface two specific receptors (p75 and trkA) with different affinities to NGF.

Signaling pathways involved in UVB-induced apoptosis may be joined with direct activation of death receptors such as CD95, in response to cyclobutane pyrimidine dimers [45]. On the other hand, UVB generates formation of ROS which induce cytochrome c release and trigger the apoptosis program in an independent way [45]. It was hypothesized that fragments of extracellular DNA (ecDNA) passing into the culture medium from apoptotic cells induced by X-rays may be one of the possible components responsible for the appearance of apoptosis in bystander cells due to binding of these DNA ligands to bystander cells via the Toll-like receptor family (TLR9) [29]. Whether this way may also operate in the UV situation is unknown. Data on the mechanism of UV-induced apoptosis in bystander cells are very limited.

POTENTIAL HAZARD OF UV-INDUCED BYSTANDER EFFECTS

Oxidative stress is an important mediator of the bystander effect induced by ionizing radiation [33], so it can be expected that oxidative stress induced in skin tissue by UV radiation will also promote the bystander effect in cells not exposed to UV, not necessarily dermal cells. Dermal tissue is built, inter alia, of two major types of cells: epithelial cells, keratinocytes and pigmented cells, melanocytes. Epithelial cells are the starting points for skin cancer, but melanocytes undergo malignant transformation to melanoma. Reactive oxygen species – superoxide radical anion, hydroxyl radical, hydrogen peroxide, singlet oxygen – induced by UV radiation [82] may lead to carcinogenesis [9]. Melanocytes are particularly sensitive to oxidative stress [37], and melanoma tissue was found to have increased levels of lipid peroxidation products as compared to benign nevi [81]. The increased locally invasive and metastatic potential of melanoma may be connected with elevated oxidative stress which could damage surrounding tissue

and thus support the progression of metastasis. Sander et al. [81] performed measurement of antioxidant enzymes (Zn- and Mn-SOD and catalase), and lipid peroxidation product (MDA) in skin biopsies of superficial spreading melanoma and age-matched benign melanocytic nevi and normal skin of young healthy controls. They found significant overexpression of antioxidant enzymes in human melanoma biopsies when compared with surrounding non-tumor tissue, benign melanocytic nevi, and young controls. Furthermore, MDA was elevated not only in melanoma cells but also in surrounding skin. Although these authors did not name these events “bystander effects” it is evident that bystander effects appeared in healthy tissue [81]. It was also observed in an animal model that increased level of oxidative stress was correlated with progression of melanoma [24]. Lipid peroxidation end-products, among them MDA and 4-hydroxy-2-nonenal (4 HNE), are not only products, but also mediators of oxidative stress [93]. This signaling molecule possesses the ability to trigger diverse cellular responses, including pro-inflammatory and anti-inflammatory reactions.

In response to UV radiation, skin keratinocytes undergo apoptosis manifested as a tan, but the cells in the area near the cells exposed to UV can also be damaged [5]. The HaCaT keratinocytes exposed *in vitro* to UV light showed a bystander effect in non-exposed cells mediated surely by molecular signals secreted into the culture medium, since medium transfer was used in the experiment. Interestingly, the medium harvested from HaCaT cells irradiated with UV (A+B) in a large dilution did not induce apoptosis, but stimulated proliferation [5], which indicates how complex the bystander effect is.

Our study of bystander effects induced by UVC radiation performed on several types of human cancer and normal cells showed not only differences in sensitivity of various cells to direct UV exposure (10 and 20 J/m²), but also in response to bystander signals inducing DNA damage, evaluated by comet assay (Kryowon²). Among the tumor cells studied, the most sensitive to UVC were malignant melanoma Me45 cells; less sensitive were HCT116 colorectal carcinoma, and K562 leukemia cells. At the same time, the strongest response to bystander signals was also shown by melanoma cells. The two normal cell lines tested, alveolar epithelial cells (BEAS-2B) and neonatal skin fibroblasts (NHDF line), showed relatively high sensitivity to UV, but responded poorly to bystander signals sent by UV-exposed cells. An example of the two cell lines' response is presented in Fig. 3.

The observed variability in response to bystander signals emitted by UV-exposed cells is in agreement with published data on the bystander response to ionizing radiation [79,90,100]. Furthermore, the bystander effect can be a reciprocal event and non-exposed cells can not only react to bystander signals secreted by irradiated cells, but also answer these signals, creating some protection against damage, e.g. micronucleus and apoptosis frequencies in exposed cells, by a reduction of ROS level [100]. Coexistence of different types of cells in dermal tissue can probably create a very complicated system which allows for mutual signaling between cells, although, up to now, with unknown consequences (protective or damaging?) for UV-induced

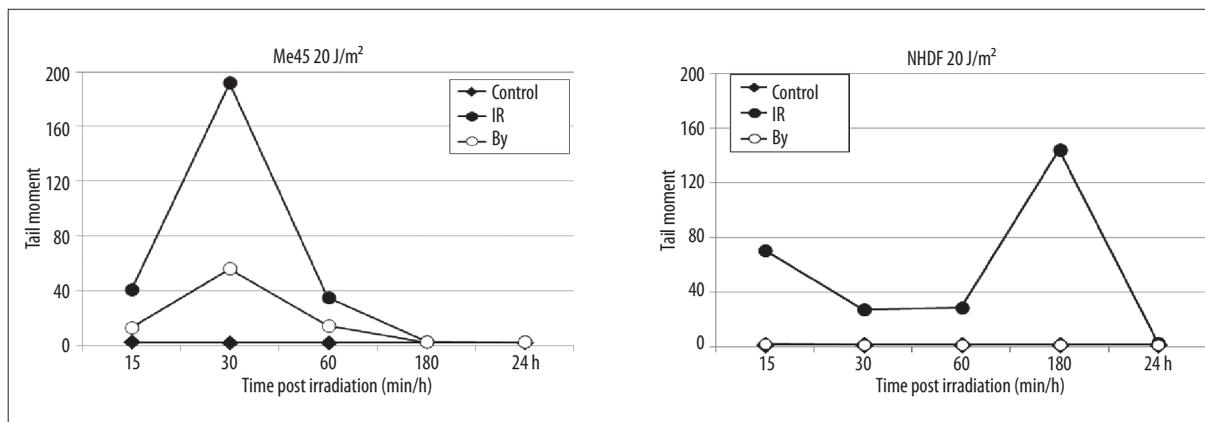


Fig. 3. Comparison of direct and bystander response to UVC radiation evaluated by alkaline comet assay in human melanoma Me45 cell line and in neonatal human dermal fibroblasts (adapted from Krzywon²)

bystander effects. Therefore an appropriate experimental system(s) is required to evaluate reciprocal communication between different types of cells exposed to UV and non-exposed neighbors.

The dominant fraction of solar radiation is UVA. It has been shown in an *in vitro* co-incubation system that only UVA, but not UVB, induced a bystander effect in human keratinocytes and fibroblasts as measured by clonogenic cell survival [97]. Single exposure to an environmentally relevant dose of UVA reduced cell survival not only in the irradiated but also in neighboring cells. Furthermore, a UVA-induced bystander effect manifested as reduced clonogenic survival of recipient cells was observed in the populations taken even after 3 days of co-incubation. That means that bystander signals generated by a single dose can persist at least for a few days after exposure and thus can enhance damaging effects of direct UVA exposure [96].

UV-induced immunosuppression may have a potential clinical implication [40]. UV radiation suppresses the immune system in several ways. For example, UVB radiation inhibits antigen presentation by Langerhans cells, increases the number of cytotoxic T lymphocytes that release immunosuppressive cytokines, e.g. IL-10 and TNF- α , and generates DNA damage that is a molecular trigger of UV-mediated immunosuppression [40]. It was shown many years ago that the incidence of skin cancer is exceptionally high in chronically immunosuppressed patients living in regions of intense sun exposure [41]. The incidence of skin cancers is also elevated among organ transplant recipients [14,68]. These observations indicate that immune surveillance plays an important preventive role in cancer generation; thus the bystander effect of UV radiation appearing as UV-induced immunosuppression can potentially lead to carcinogenesis.

It is however worth noting that a UV-induced bystander effect can sometimes have a protective, rather than damaging effect [65]. It was found in B16 melanoma cells in transfer medium experiments that both UVA and UVB induced melanogenesis in bystander cells. Melanin, which is generated by melanocytes and transported to keratinocytes, is an important natural agent which protects human skin from UV radiation damage. Especially eumelanin has protective properties due to its resistance to degradation and its ability to scavenge reactive oxygen species. [13]. On the other hand,

however, melanin enhanced generation of ROS by ultraviolet A radiation, whereas it protected melanocytes from direct DNA damage by ultraviolet B radiation [46]. This is probably connected with interference of melanin with DNA repair [95]. In experiments performed by Nishiura et al. [65], the increase of melanogenesis was accompanied by a significant decline in the level of mitochondrial membrane potential in the UVA-irradiated bystander cells, a few hours after medium transfer, but no such decline was seen in the UVB-irradiated bystander cells. Furthermore, in UVA experiments a significant increase of intracellular ROS was measured in bystander cells, whereas there was no such increase of ROS in UVB bystanders. These results indicate that the mechanism of melanogenesis induced through the bystander effect is different for both UV spectra. It was further found that increased levels of ROS in UVA bystander cells resulted from a decline of the mitochondrial membrane potential due to an influx of calcium ions into the cells [65], the effect resembling that observed in ionizing radiation-induced bystander cells [51]. It is however an unanswered question whether normal melanocytes can respond to a UV-induced bystander effect by melanogenesis, similarly as B16 melanoma cells.

CONCLUSION

Ultraviolet radiation has, in addition to a beneficial effect for the body, a serious adverse effect. UV radiation operates in dependence on wavelength through direct DNA damage (CPD and 6-4 PP in UVB and UVC) leading to mutagenesis, and induction of reactive oxygen and nitrogen species (mainly UVA). ROS and RNS in UV-exposed cells and in bystander cells contribute to damage such as nucleotide oxidation, gene mutation, genomic instability, apoptosis, inflammation and immune suppression. The oxidative damage to DNA and inflammatory response may lead to carcinogenesis. Although the bystander effect after UV radiation has been recognized in cell culture systems, its occurrence *in vivo* has not been studied. UV is the main factor inducing skin cancers such as basal and squamous cell carcinomas, and malignant melanomas. However, it is reasonable to expect that oxidative stress and damage to DNA and lipid structures induced in UV-exposed skin tissue can trigger signaling pathways leading to genomic instability and a damaging bystander effect in surrounding tissues and in circulating blood cells that in consequence can increase the harmful and even

carcinogenic potential of UV radiation. However, further studies are required, specifically looking for activation of various cell signaling pathways in more composed systems, e.g.

containing different types of dermal cells coexisting in culture *in vitro* (3D system, artificial skin), or in animal models, since mutual signaling is important for the bystander effect.

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