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Intercellular Communication in Response to Radiation Induced Stress: Bystander Effects in Vitro and in Vivo and Their Possible Clinical Implications

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1. Introduction

Communication between cells is important for maintaining homeostasis, the physiological regulatory processes that keep the internal environment of a system in a constant state. A disease can disturb the internal equilibrium of cells, and this can be further disrupted by various therapies. Malignances are the diseases that need to be treated by highly aggressive methods, such as radiotherapy, which affects not only tumor cells but also normal cells adjacent to the tumor and usually included in the radiation field. This treatment may interfere with normal intercellular communication. It has been a central radiobiological dogma for decades that damaging effects of ionizing radiation are the result of direct ionization of cell structures, particularly DNA, or are due to indirect damage *via* water radiolysis products. Indeed, DNA damage such as chromosomal aberrations, micronuclei, sister chromatid exchange and mutagenesis result from ionizing radiation. All of these types of damage, if unrepaired, can lead to cell death or, if misrepaired, can lead to genomic instability and carcinogenesis. Recently however, the attention was focused on the third mechanism, a phenomenon termed "radiation induced bystander effect" (RIBE). This phenomenon is a non-targeted effect where molecular signal(s) produced by directly irradiated cells elicit subsequent responses in unirradiated neighbors. These responses are manifested as decreased survival, increased sister chromatid exchanges (SCE), chromosomal aberrations (CA), micronucleus (MN) formation, gene mutations, apoptosis, genomic instability, neoplastic transformation and a variety of damage-inducible stress responses (reviewed in Morthersill and Seymour, 2001, Lorimore et al., 2003, Morgan, 2003a, 2003b, Little, 2006a,b, Chapman et al. 2008, Rzeszowska-Wolny et al., 2009a). Bystander effect accompanies very low doses of alpha particles (mGy and cGy), (Nagasawa and Little, 1992, Lorimore et al., 1998), as well as irradiation of cells with a low LET radiation (X- and gamma rays), even at conventionally used higher clinical doses (Morthersill and Seymour, 1997, 1998, 2002b, Przybyszewski et al., 2004). The mechanisms responsible for RIBE are complex and not quite well-known. Mechanisms by which bystander signals may be transmitted from irradiated to non-irradiated cells involve direct cell-to-cell contact mediated by gap

junction intercellular communication (GJIC), and indirect communication by means of soluble factors secreted by irradiated cells into the surrounding medium. It is believed that molecular signaling factors released by cells irradiated and dispatched to the medium or transferred through GJIC induce various signaling pathways in neighboring cells, leading to the observed effects. The nature of these factors may be different and they have not been definitely defined. In addition to short-lived oxygen and nitrogen free radicals (Matsumoto et al., 2001, Azzam et al., 2002), long-lived radicals (Koyama et al., 1998), interleukin 8 (Narayanan et al., 1999), TGF-β (Shao et al., 2008 a, b, Massague and Chen, 2000) and other agents can be included. Potentially, bystander phenomenon could play an important role in the appearance of undesirable localized or systemic radiotherapeutic effects in tissues not included in the irradiation field. Furthermore, the effect may appear after low-dose irradiation during diagnostic radiology procedures and following application of a radioisotope for diagnosis or treatment (Prise and O'Sullivan, 2009). Factors emitted by irradiated cells may have impact on risk of genetic instability and the induction of mutation. However, the radiation-induced bystander effect may have both detrimental and potentially beneficial consequences. If cells directly hit by ionizing energy will, through their signals (secreted or transmitted through the gap junction) damage adjacent cancer cells, or will initiate differentiation of these cells, it is desirable. However, if normal cells are damaged (epithelial and endothelial cells, fibroblasts, leucocytes, etc.), then the effect may be a disadvantage that increases the unwanted effects of radiotherapy such as late complications and second primary tumors. Bystander effect can be particularly important in the case of the use of current techniques of irradiation, such as 3D conformal radiation therapy (3D-CRT) and intensively modulated radiotherapy (IMRT), the purpose of which is to reduce the irradiation dose in healthy tissues (Followill et al., 1997). Some data indicate that bystander effect also occurs *in vivo* (Koturbash et al., 2006, 2007, Ilnytskyy et al. 2009). The studies of bystander effect in *in vivo* animal models show that the post- radiation damage can appear in tissues distant from the place of irradiation, and the effect may vary depending on the type of tissue. However, recent experimental results (Mackonis et al., 2007), including our own (Widel et al. 2008, and unpublished), show that cross-talk between irradiated and un-irradiated cells may be sometimes protective and non-irradiated cells, which are in the vicinity of irradiated cells can hamper the effects caused by their irradiation. Furthermore, a radioprotective bystander effect has been observed in several studies with low-dose exposure in the form of increased cell redioresistance to subsequent higher doses (e.g. Sawant et al., 2001, Prise et al., 2006). Less known are the consequences of bystander effect in the case of dose fractionation during external irradiation. Our preliminary results from *in vitro* fractionation dose experiments, presented in this Chapter indicate that apoptosis is even more effectively induced in human melanoma radiation-targeted and bystander cells when the same dose is delivered in 3 fractions than in one single dose. A growing body of experimental *in vitro* and *in vivo* data indicate the occurrence of bystander phenomenon in radionuclide-based radiotherapy (Xue et al., 2002, Gerashchenko and Howell, 2004, Boyd et al., 2006, Mairs et al. 2007). However, studies of radionuclide-induced bystander effect demonstrate varying responses (compared to low LET radiation-induced ones), being either damaging or protective depending on dose and type of emitters. The practical consequences, as well as capacities of the bystander effect, in terms of modulating radiotherapeutic approaches, are therefore still uncertain and are the subject of intensive research. It is possible that the impact of bystander signaling on both cancer and healthy tissue responses is more relevant than it is believed at present. Below is a comprehensive

review of the various aspects of radiation-induced bystander effect, based on the current knowledge and our own experimental results.

2. History of bystander effect phenomenon

First observations of the bystander effect phenomenon appeared in the nineties of the last century. Using a low-dose of alpha particles which targeted only 1% of cultured Chinese hamster ovary cells (CHO), Nagasawa and Little (1992) noticed cell damage in the form of sister chromatid exchanges (SCE) appearing in about 30% of cells. The level of damage increased with 0.3-2.5 mGy dose, but not with higher ones. Subsequent experiments showed an increase in the number of cells with overexpression of *TP53* gene after 6 mGy alpha irradiation, but not after exposure to the same dose of X-rays (Hickman et al., 1994). Very soon, it appeared that this effect also occurs in cells exposed to radiation with a low LET radiation. It was observed that the factors inducing the observed effects in non-irradiated cells are soluble and can be passed through the growth medium (Deshpande et al., 1996, Morthersill and Seymour, 1997), or by an intercellular connection slot (Azam et al., 1998). Morthersill and Seymour (1997) showed that factors present in the culture medium collected from epithelial cells exposed to gamma radiation decreased survival of clonogenic non-irradiated cancer and epithelial cells in culture; therefore for the bystander effect to occur the contact of irradiated cells with non-irradiated is not necessary. Furthermore, reduced cell survival did not occur when medium harvested from irradiated fibroblasts was used. The cytotoxic effect of irradiation-conditioned medium (ICM) has been observed in several experimental systems following both particle (Deshpande et al., 1996, Lorimore et al., 1998) and photon irradiation (Clutton et al., 1996, Matsumoto et al., 2001). It was found that the bystander effect-signaling molecules may include tumor necrosis factor beta (TGFβ) and interleukin-8 (Narayanan et al., 1999) secreted to the medium or transferred through GJIC. Closing these connections by *lindane*, an inhibitor of gap junction, lead to the inhibition of bystander effect, evidenced as the reduced expression of *TP53*, *CDKN1A* (p21) and *CDC2* genes (Azzam et al., 1998), or increased survival of clonogens (Bishayee et al., 1999). Several studies have demonstrated that the radiation-induced bystander effect triggers apoptosis (Prise et al., 1998, 2006, Morthersill and Seymour, 2001, Przybyszewski et al., 2004) and increase of micronucleus frequency, DNA double-strand breaks (DSBs) measured as histone H2AX phosphorylation (Sokolov et al., 2007, Burdak-Rothkam et al., 2007), accumulation of p53 (Tartier et al., 2007) and ATM and ATR proteins (Burdak-Rothkam et al., 2008), epigenetic changes, such as DNA hypomethylation, as well as the expression of other genes (Chaudry, 2006, Iwakawa et al., 2008, Rzeszowska-Wolny et al., 2009b). Many of these experiments showed that higher doses of radiation, including those used in conventional radiotherapy, also induce bystander effects in non-irradiated cells. They confirmed the quantitative biophysical model of Nikjoo and Kvostunov (2003, 2006) which assumes that RIBE may be a component of neighborhood responses to radiation, both at low and high doses. The results obtained in tissue explant culture (Belyakov et al., 2002, 2006, Mothersill and Seymour, 2002b), tri-dimensional cell culture, *in vivo*-like models (Bishayee et al., 1999, 2001, Belyakov et al., 2005), and in animal studies (Koturbash et al., 2006, 2007, 2008) all point out to the bystander phenomenon relevance to clinical radiotherapy. Therefore, one cannot exclude that the intensity of side effects in healthy tissues following fractionated radiotherapy may be partly related to bystander effect. It is suspected that this effect may also lead to genetic instability, the consequence of which can involve development of

secondary cancers (Hendry, 2001). Not always, however, radiation induced bystander effect has a damaging action. The signals emitted to the microenvironment by irradiated cells seem to induce in cells unexposed to radiation more complex effects, inter alia their differentiation, probably as a comprehensive response in order to preserve the integrity of the tissue (Belyakov et al., 2006, Vines et al. 2009).

3. Radiation induced bystander effect, genetic instability and adaptive response

Bystander effect, genetic instability and adaptive response seem to be related. Known as the genetic instability are the delayed effects such as lethal mutation, unstable chromosome aberrations, and delayed reproductive death (DRD) in distant generations of cells previously exposed to radiation (Gorgojo et al., 1989, Mendonca et al. 1989), or arising *de novo* chromosome aberrations (Kadim et al., 1995, Marder and Morgan 1993, Weissenborn and Streffer, 1989) and gene mutations (Little et al., 1997). Delayed reproductive death (DRD), manifested as diminution of clonogenic cell survival, appears to be caused neither by apoptosis nor by necrosis. DRD is mainly observed in cells with uninterrupted mechanisms of DNA double-strand breaks repair (Little et al., 1990, Little, 1999), but is not observed in cells with impairment of these mechanisms (Chang and Little, 1992). It was demonstrated that cell clones with post-radiation genetic instability evolve through many generations of descendants, the cytotoxic factors affecting non-irradiated cells (Kadim et al., 1995) and, the effect being independent of intercellular gap junctions (Nagasawa et al., 2003). Studies of genetic instability in which only some mouse marrow stem cells were targeted by alpha particles showed higher numbers of cells with chromosome aberrations than those of irradiated cells. These lesions are transferred to the descendant cells forming colonies (Loroimore et al., 1998). In addition, the surviving fraction of clonogenic cells decreases deeper with the dose than would result from the dose absorbed, provided the damage resulted from communication of lethally-irradiated cells with non-irradiated cells. Increased mutation frequency of hypoxanthine-guanine-phosphoribosyl transferase gene (*HPRT*) in distant generations of murine hematopoietic stem cells irradiated *in vitro* with both the X-rays and neutrons was also observed (Harper et al., 1997). Furthermore, human T-lymphocytes showed chromosome aberrations transferred through generations of their progenitor cells that had been irradiated with 3Gy X-rays dose (Holmberg et al., 1995). Factors inducing the bystander effects can be passed through gap junctions (Zhou et al., 2000, Azzam et al., 2002], or secreted to the surroundings (Lyng et al., 2000, Morthersill and Seymour, 1998). Some of them are clastogenic and can induce chromosomal damage in non-irradiated cells, analogous to that in directly-hit cells. Huang et al. (2007) observed that growth medium conditioned by some chromosomally unstable RKO derivatives induced genomic instability, indicating that these cells can secrete factor(s) that elicit responses in non- irradiated cells. Furthermore, low radiation doses suppressing the induction of delayed genomic instability by a subsequent high dose, are indicative of an adaptive response for radiation-induced genomic instability. Adaptive response is a phenomenon by which cells irradiated with a sub-lethal radiation dose (mGy or cGy) may become less susceptible to subsequent high-dose (a few Gys) radiation exposure (Wolff, 1996, Marples and Skov, 1996). The mechanism of this phenomenon is not sufficiently known. Irradiation leads to disturbances of the balance between pro-oxidant and anti-oxidant signaling molecules; one of such molecules can be nitric oxide (NO) (Spitz et al., 2004). An increase of radioresistance

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was observed in human glioblastoma A-172 cells with functional *TP53* gene when they were co-incubated with irradiated (1-10 Gy X-rays) cells of the same line transfected with mutated *TP53* gene (A-172/mp53), or incubated in the presence of conditioned medium from irradiated cells (Matsumoto et al. 2001). The sign of radioresistance was the accumulation of HSP72 and p53 protein which had declined in the presence of nitrogen oxide scavenger or inducible nitrogen oxide synthase inhibitor. Another probable mechanism thought to be a cellular adaptive response is the low-dose enhancement of DNA repair ability and antioxidant activity, resulting in more proficient cellular responses to the subsequent challenge. Sawant et al. (2001) observed that the exposure of C3H 10T91/2 cells to single alpha particle radiation, which hit only 10% of cells, caused the death of a much larger number of cells. However, the use of 2cGy gamma rays 6 hours before exposure to the alpha particles continuously reduced the bystander effect expressed as increased surviving cell fraction. Increased resistance induced by large dose of gamma radiation was also observed in cells of the same line if they were pre-exposed to a cGy dose of 60-Co (Azzam et al., 1996), and the reduction in the percentage of micronuclei was accompanied by an increase in the repair of DNA double-strand breaks (Azzam et al. 1994). Recently, it was presented that different cell lines can show different pattern of response to low priming dose (Ryan et al. 2009). An adaptive response was detected in cell lines known to produce hypersensitive response, and was inversely correlated with the bystander effect suggesting that an adaptive response may be mutually exclusive to the bystander effect.

4. The mechanisms of radiation induced bystander effect

The ionizing radiation acts through direct ionization of organic macromolecules or through reactive oxygen species (ROS), namely, hydroxyl radical (OH$^\bullet$), hydrogen peroxide (H$_2$O$_2$) and superoxide radical anion (O$_2^{\bullet-}$), the effect of which is primarily oxidative DNA damage (Marnett, 2000, Matsumoto et al., 2007). Half-life of ROS is extremely short and penetration distance is expressed in micrometers. Therefore, these factors may not reach non-irradiated cells. Electron spin resonance studies have shown, however, that long-lived radicals with a period of half-lives ca. 20 hours may appear in cells after irradiation, even at room temperature (Koyama et al., 1998); if transferred to the surroundings, they may be the factors inducing DNA damage in non-irradiated cells. The long-lived secondary radicals are likely to be less active in damaging DNA than the extremely active primary radicals generated during irradiation time. Therefore, DNA damage induced by secondary radicals may not be a sufficient barrier to stop the replication of DNA and can lead to duplication of altered DNA through generations of cells, and finally to mutation and neoplastic transformation (Azzam et al., 2003, Clutton et al., 1996, Iyer and Lehnert, 2000, Lala and Chakraborty, 2001). DMSO, a radical scavenger, reduced the level of DNA damage in irradiated cells and inhibited the bystander effect which seems to confirm the role of reactive forms of oxygen in initiating signaling molecules (Hussain et al., 2003, Kashino et al., 2007). Also, the use of vitamin C as a scavenger of long-lived radicals compromised the level of micronuclei in human fibroblasts co-incubated with irradiated cells (Harada et al., 2008), as well as in K562 myelogenous lukemia cells treated with medium from irradiated cultures of the same cell line collected one hour post irradiation (Konopacka and Rzeszowska-Wolny, 2006). However, not only DNA is the target for ROS; no less important are the fatty acid molecules, in which the peroxidation chain reactions lead, through short-lived lipid radicals, to stable end-products such as malondialdehyde (MDA), 4-

hydroxynonenal (4HNE) and other with mutagenic and carcinogenic properties and which can form massive DNA adducts (Marnet, 2000, Zhong et al., 2001). The end-products of lipid peroxidation have secondary signaling molecule properties and can activate a cascade of signals leading to either DNA damage repair, or to damage stabilization or apoptosis (Hu et al., 2006). In our research we found increased MDA concentration in irradiated Me45 human melanoma cells growing in the form of megacolonies, as well as in the neighboring megacolonies growing in the same flask but protected against irradiation with a lead shield (Przybyszewski et al., 2004). At the same time, we found in both the irradiated and shielded megacolonies, decreased glutathione peroxidase (GSH-Pox) and mitochondrial superoxide dismutase (MnSOD), as well as elevated numbers of single- and double-strand DNA breaks (SSBs and DSBs), as assessed by single cell gel electrophoresis. The level of DNA breaks in non-irradiated cells was lower and appeared with several-hour delay compared to that observed in irradiated cells, which may suggest participation of long-lived radicals in the bystander effect induction (Przybyszewski et al., 2004). Time-shifted appearance of DSBs in neighboring cells estimated as the expression of phosphorylated histone H2AX (γH2AX foci) has been observed in the *in vitro* (Hu et al., 2006, Sokolov et al. 2007) as well as in *ex vivo* (Sedelnikova et al., 2007) conditions. While the phosphorylation of histone H2AX at serine 139 is a very early-stage event in cells directly exposed to radiation, the appearance of gamma-H2AX foci in cells co-cultured with irradiated ones, or treated with ICM only, may even take several hours. The gamma-H2AX foci, which indicate the presence of DNA DSBs in cells exposed to the signals transmitted by irradiated cells, co-localize with other proteins involved in the cell cycle control and DNA damage repair, such as ATM, MRE11, NBS1, Rad50 and 53BP1 (Sokolov et al., 2007). It is worth noticing that, based on ATM foci enumeration, Ojima et al. (2009) found that DSBs induced by the radiation-induced bystander effect persist for long periods (over 24 h), whereas DSBs induced by direct radiation effects are repaired relatively quickly. However, ATM foci persisted even longer (48 h) if bystander fibroblasts were co-incubated with very low (1.2 mGy) irradiated counterparts. This indicates that bystander signals coming from irradiated cells *induce* chromatin damage which differs from that induced by direct irradiation. It has been shown that not exclusively irradiation of DNA but irradiation of cytoplasm induces cytogenetic damage in both irradiated and bystander glioma cells and fibroblasts to a comparable extent (Shao et al., 2004) The bystander responses were completely eliminated when the populations were treated with nitric oxide scavenger or agent which disrupt membrane rafts. This finding shows that direct DNA damage is not required for induction of important cell-signaling mechanisms after low-dose irradiation and that, the whole cell should be considered a sensor of radiation exposure. The use of compounds that compromise the level of nitrogen oxide abolishes the bystander effect elicited as γH2AX expression. Nitric oxide (NO) seems to be an important signaling molecule transmitted by irradiated cells, which initiates the changes in cells not exposed to radiation (Matsumoto et al., 2001, 2007, Shao et al., 2008a, b). This small molecule is also a free radical which is synthesized from the L-arginine with the participation of nitric oxide synthase (NOS). It plays important, often contradictory roles in many biological processes, stimulating either the proliferation or apoptosis, which primarily depends on its concentration (Shao et al., 2008b). Nitric oxide is vasodilatator, neurotransmitter and an immunomodulatory agent, but it may also cause damage to DNA by generating peroxynitrite anion (ONOO-), which may cause oxidation or nitration of DNA (Xu et al., 2002). Shao et al. (2008a, b) demonstrated that radiation-generated NO induced in glioma cells TGFβ1, the multifunctional transcription factor

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involved in the transcription of proteins engaged in cell proliferation and differentiation, immunomodulation, cell-cycle control and apoptosis (Massague and Chen, 2000). The use of inducible nitric oxide synthase inhibitor, or anti-TGF antibodies which compromise micronuclei in cells directly irradiated with alpha particles and adjacent non-irradiated cells indicates a positive feedback. However, NO role as a mediator of the bystander effect has not been observed in all tested glioma cell lines (Matsumoto et al., 2001). In several types of cancer (colon, lung, throat) expression of inducible nitric oxide synthase (iNOS) was also linked to the *TP53* gene mutation (Lala and Chakraborty, 2001) indicating that the correct protein of p53 gene may negatively regulate the accumulation of iNOS. Many other factors were proposed as the bystander effect mediators, among them interleukin 8 (Narayanan et al., 1999), soluble tumor necrosis factor (TNFα) as well as Fas and TRAIL death ligands (Lucen et al. 2009). Also, multiple pathways are activated that take part in transmitting the bystander effect signals. Those induced in human fibroblasts by alpha particles (0.3-3 cGy) and transmitted through the GJIC or surrounding environment activated in adjacent cells various proteins such as MAP- kinase, NFκB, Raf-1, ERK1/2, JNK, AP-1 and others (Azzam et al. 2002, Lyng et al., 2006). Since application of SOD and catalase neutralizes the resulting oxygen radicals and hydrogen peroxide and hampers the bystander effect (reduction in the level of micronuclei, inhibition of nuclear factor κB and p38 MAPK activation), the mediators of these processes appear to be reactive oxygen and nitrogen species (Azzam et al., 2002). Targeting the nucleus or cytoplasm of HeLa cells by single helium ions induced expression of 53BP1, the protein which marks double-stand breaks in DNA (Tartier et al., 2007). The use of aminoguanidine, an inducible NO synthase inhibitor, or radical scavenger DMSO, cause inhibition of 53BP1 protein expression in both irradiated and co-incubated non-irradiated cells, pointing to the NO and ROS as the mediators of these lesions. At the same time, it was observed that antibiotic *filipin*, which damages the glycosphingolipid microdomains in cellular membrane, inhibited cellular signals from irradiated cells and led to a drastic reduction in the 53BP1 foci in neighboring cells. This reveals that transmission of bystander signals is dependent on the integrity of the cellular membranes, whereas membrane integrity was not necessary to generate the damage in irradiated cells. Also, the presence of mitochondria was necessary to generate bystander signals by irradiated cells, but was not necessary to their reception (Tartier et al., 2007). Calcium ion channels seem to play a role in the transmission of bystander signals. It was observed that biogenic amines, such as serotonin (5-hydroxytryptamine, 5-HT) and dopamine, may be the transducers of signals emitted by irradiated cells. The level of 5-HT neurotransmitter in culture medium decreased after irradiation of cells, likely due to its binding to the receptors which form the calcium channels, and leads to increased level of micronuclei (Poon et al., 2007). These effects were abolished after treatment of cells with calcium channel blockers calcicludin or rezerpin, which are the natural antagonists for serotonin (Poon et al., 2007, Shao et al., 2006). The study of transcript levels using DNA microchips may indicate signaling pathways and genes that are involved in the radiation-induced bystander effect. Gandhi et al. (2008), when examining the overall gene expression (global genome expression), after irradiation of human lung fibroblasts with alpha particles (0.5 Gy and 4-hour co-incubation with non-irradiated cells), observed that the expression of over 300 genes in both groups (hit and non-hit) was changed, and that 165 genes were common to both groups. Among them were genes mainly over-expressed in irradiated cells (CDKN1) and those that were over-expressed equally in irradiated and neighboring cells, namely NFκB–regulated PTGS2 (cyclooxygenase 2), IL8 and BCL2A1. However, Chaudhry (2006) observed that gene

expression profile differs in irradiated human fibroblasts and in non-irradiated cells treated only with radiation-conditioned medium. In the former, over-expressed were the genes of early response to radiation, while in the bystander cells the over-expressed ones included genes involved in the intercellular communication. In our genome-wide microarray study, we compared transcript profile changes in Me45 human melanoma cells grown in culture medium from irradiated cells with those which occurred after irradiation and we also observed the bystander effect at the genome level (Rzeszowska-Wolny et al., 2009). Using the criterion of a greater than ±10% change, transcripts of >10,000 genes were shown to be expressed at increased or decreased levels under both conditions, and almost 90% of these were common to ICM-treated and X-rays-treated cells. Among them were genes involved in the neuronal receptor-ligand interactions, oxidative phosphorylation, cytokine–cytokine receptor interactions, proteasomes, ribosomes and cell cycle regulation. All these tests indicate a very complex mechanism of cell response to both ionizing radiation and for signals transmitted by them to communicate with the neighboring cells.

5. The role of the p53 protein in the response to bystander signals

The *TP53* gene is a tumor suppressor gene which participates in the regulation of cell cycle and apoptosis. Its main role is to prevent the transmission of genetic disorders in cells to daughter cells by extending G1 phase, which allows the cell to repair DNA damage induced by various egzo-and endogenous agents, mainly the oxidative stress. When the damage is too bulky or the repair is ineffective, *TP53* initiates apoptosis through its own product, p53 protein, which is a transcription factor for multiple genes involved in DNA repair, regulation of cell cycle and apoptosis (Chipuk and Green, 2006, Tlsty, 2002). The role of p53 protein in the bystander effect is debatable, however. Research carried out using human fibroblasts cell lines, where only a small fraction of cells was exposed to alpha particles has shown a significant increase in p53, as well as p21[Waf1] protein, not only in the targeted, but also in the non-targeted cells (Azzam et al., 1998). The effect disappears after inhibition of the gap junction intercellular communication. Similarly, expression of p53 protein was observed in the rat lung epithelial cells adjacent to alpha particle-targeted cells (Hickman et al, 1994). However, survival of clonogenic fibroblasts after 2 and 4 Gy was increased when they were exposed to the medium from fibroblasts gamma-irradiated with a dose of just 1cGy. This was accompanied by the reduction of p53 protein level in addition to the increase in intracellular pool of reactive oxygen radicals and DNA-repair protein nuclease APE (Iyer and Lehnert, 2000). The appearance of DSBs is accompanied by DNA binding protein 53BP1 which may be detected immunochemically using fluorescent-labeled antibodies. It was shown that the irradiation of cell cytoplasm with single alpha particles, induced increased numbers of 53BP1 foci not only in nuclei of irradiated cell, but also in adjacent to them non-irradiated cells (Tartier et al., 2007). The use of inhibitors targeting reactive oxygen radicals and nitric oxide prevented the formation of DNA breaks in irradiated and adjacent cells. This indicates that the bystander effect signals are transmitted not only between cells but even between cell compartments. Also, the use of membrane specific antibiotic (*filipin*) to disrupt membrane-dependent signaling has resulted in lowering the number of clusters of 53BP1 foci an important sensors of DNA double strand breaks, in cells co-incubated with irradiated ones, indicating that reception of bystander effect signaling molecules requires the integrity of the cellular membranes (Tartier et al., 2007). The tests in rats which were given 1Gy doses of X-rays, both whole-body or head-area-only, revealed expression of p53

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protein in the spleen of animals, pointing to the involvement of the TP53 gene in the bystander effect *in vivo* (Koturbash et al., 2008). However, in our own research using HCT116 colon cancer cells lines differing in *TP53* status, and the transwell system of co-cultivation, we observed that *TP53 gene* is not required to uncover the bystander effect. Non-irradiated *TP53*-knockout cells (HCT116p53 -/-) were even more sensitive to apoptosis induced by signals sent by irradiated (2 Gy) cells than wild-type cells (HCT116p53+/+) (Widel et al., 2009). In the same experiments we noticed that the level of micronuclei induced in cells co-cultured with non-irradiated ones did not differ between both lines. Recently, He et al (2010) found that the bystander effect after irradiation can be modulated by the p53 status of irradiated hepatoma cells and that a p53-dependent release of cytochrome c may be involved in the RIBE. Following irradiation cytochrome c was released from mitochondria into the cytoplasm only in HepG2 (wild-type p53) cells, but not in PLC/PRF/5 (p53 mutated) or Hep3B (p53-null) cells. Only irradiated HepG2 cells induced bystander effect elicited as micronuclei (MN) formation in the neighboring Chang liver cells. In conclusion, the various criteria for assessing the role of *TP53* gene reveal differences in its response to bystander effect signals.

6. Bystander effect can function bi-directionally

Recent studies have shown an interplay between adjacent irradiated and non-irradiated cell populations. Thus, signals leading to damage in non-irradiated cells, sent by the irradiated ones, are answered by non-hit cells affecting in turn the directly-irradiated ones. Experiments performed on MM576 melanoma cells, the goal of which was to investigate the impact of modulating irradiation fields in a way to resemble the intensity-modulated radiotherapy technique (IMRT) on survival showed, that the mutual communication works in three different manners (Mackonis et al., 2007). The first type of this communication, the classic "bystander effect", occurs when irradiated cells growing in one part of the field damage the adjacent non-irradiated cells growing in another part of the field. The second type of communication, causes an increase in the survival of non-irradiated cells, when they are co-cultured with cells exposed to high doses (6-20 Gy) or even a lethal dose. One of the factors responsible for this process is, according to these authors, the eruption of "death-burst signals", which promotes proliferation of the non-irradiated cells, although the authors do not specify the chemical nature of these signals. The third type of communication causes increased survival of cells that have received a high dose of radiation, through signaling from neighboring cells exposed to low-dose in another part of the field (Mackonis et al., 2007). Also, the irradiation of human fibroblasts with low doses of alpha-particles resulted in an increased proliferation, reduction of the level of p53 and CDKN1 (p21Waf-) proteins and an increase in the level of the CDC2 kinase. The promitogenic effect was associated with an increase in the level of the TGFβ1-induced by reactive oxygen species (Iyer and Lehnert, 2002). Our recent study revealed bystander effect of the third type, similar to that described by the Mackonis, indicating the bilateral signaling of irradiated and non-irradiated cells (Widel et al., 2008, and unpublished). Using the transwell system of co-incubated irradiated mouse lung cancer cells (LLC) with non-irradiated fibroblasts (NIH3T3) growing in inserts we studied the mutual interaction of cells in terms of micronuclei and apoptosis induction. The membrane of insert bottom with 0.4 μm pores separates both types of cells but enables free circulation of medium between them. LLC cells growing in 6-well plates were irradiated with doses of 2 and 4 Gy X-rays generated by a therapeutic accelerator (Clinac 600). Immediately after irradiation the inserts with non-irradiated (bystander) fibroblasts were

inserted into the wells and co-incubated for a desired time. Another set of irradiated LLC cells was incubated without cells in inserts, the latter filled with medium only. Micronuclei and apoptosis were scored in microscopic slides prepared from cells harvested at different time-points. The results show that the irradiated cells induced apoptosis and micronuclei in bystander fibroblasts. For the first time we show the radioprotective effect of normal cells on irradiated cancer cells (the opposite bystander effect); thus the percentage of micronuclei and apoptosis in irradiated LLC cells co-incubated with NIH3T3 fibroblasts was significantly decreased in comparison with analogous levels in the irradiated LLC cells incubated without fibroblasts growing in inserts (Figure 1).
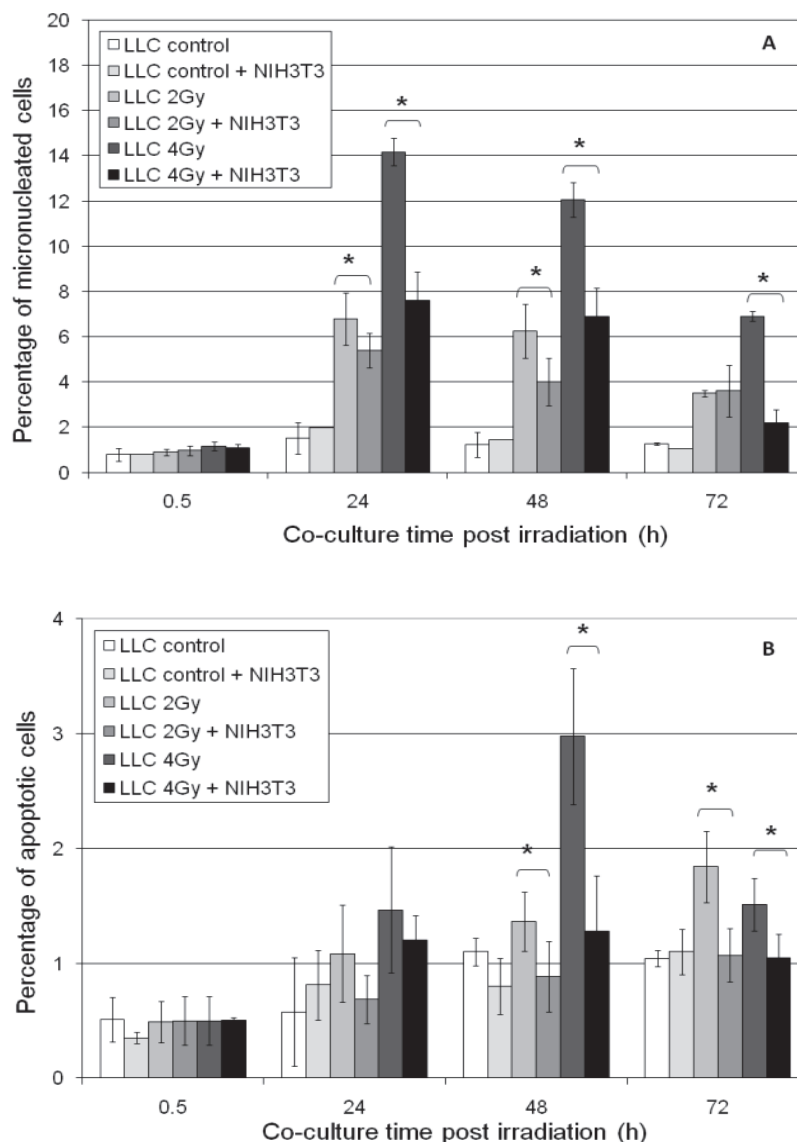

Fig. 1. Non-irradiated murine NIH3T3 fibroblasts co-cultured with irradiated Lewis lung carcinoma cells significantly diminish micronuclei (A) and apoptosis frequency (B) in irradiated (2 and 4 Gy) cancer cells compared with those irradiated and incubated without fibroblasts. Results are means ± standard deviation from three independent experiments (*p<0.05, Student's t-Test).

The mechanism of this phenomenon requires clarification. It seems that the radioprotective bystander effect is a feature of normal fibroblasts. Indeed, the same effect, i.e. a significant reduction in the level of micronuclei and apoptosis in irradiated human melanoma Me45 cells was observed when these were co-incubated with normal human fibroblasts (Widel et al., unpublished). The progressive increase of micronuclei and apoptosis was paralleled by an increase of ROS; however, the ROS level in irradiated melanoma cells, which were co-cultured with fibroblasts, was significantly diminished. Such a radioprotection was not observed in irradiated Me45 cells co-cultured with cells of the same line of melanoma (Widel et al, unpublished). We believe that the observed radio-protective effect of non-irradiated fibroblasts exerted on irradiated melanoma cells may result from signaling molecule(s) modifying the redox status of irradiated cells. Similar effect is likely to occur during cancer radiotherapy, causing some decrease of damage to cancer cells owing to fibroblasts present in tumor tissue.

7. Fractionated irradiation and bystander effect

Experimental data on bystander effect mostly come from single-dose application experiments *in vitro*. However, there is a lack of knowledge, which would have potential clinical implication, e. g. whether bystander effect occurs during fractionated treatment. Mothersill and Seymour (2002a) performed experiments involving repeated treatment of bystander cells with medium collected from irradiated cells as well as involving repeated dose exposure of cells producing bystander signals, as a way of mimicking fractionated exposures. The recovery factor was defined as the surviving fraction of the cells receiving two doses (direct, or ICM) separated by an interval of 2 h divided by the surviving fraction of cells receiving the same dose in one exposure. The authors observed that fractionated bystander treatments removed the effect of dose sparing that is observed after conventional fractionated regime, during which cells can repair DNA damage. Using Me45 human melanoma cell line established at the Center of Oncology in Gliwice (Kramer-Marek et al, 2006) we compared frequency of apoptosis and micronuclei formation in directly irradiated and bystander cells after single doses (1.5 - 6 Gy) and after doses divided into 3 fractions given at consecutive days (3 x 0.5 Gy – 3 x 2 Gy). We used a transwell system of co-incubation which allows co-culturing the irradiated cells growing in wells with non- irradiated cells growing in inserts. This system to some extent resembles situation *in vivo*, due to prolonged contact of non-irradiated and irradiated cells. As a source of X-rays (6 MV) Clinac 600 therapeutic accelerator was used. Non irradiated control cells were-sham exposed. After irradiation, inserts with growing non irradiated cells were placed into wells with irradiated ones and co-incubated. Before irradiation medium in both, wells and inserts, was replaced by fresh aliquots. To observe the response of hit and bystander cells after the set time of incubation (0, 24 and 48 h), we performed microscopic analysis of micronuclei induction and apoptosis. The results obtained show that both single dose irradiation and fractionation of the dose into three fractions effectively induced bystander effect in malignant Me45 melanoma cells. However, fractionated irradiation at low doses (Fig. 2) appears to be much more effective in inducing micronuclei in directly hit and bystander cells, whereas higher apoptosis induction was clearly seen in hit, and especially in bystander cells, at all doses in fractionated system (Fig. 3).
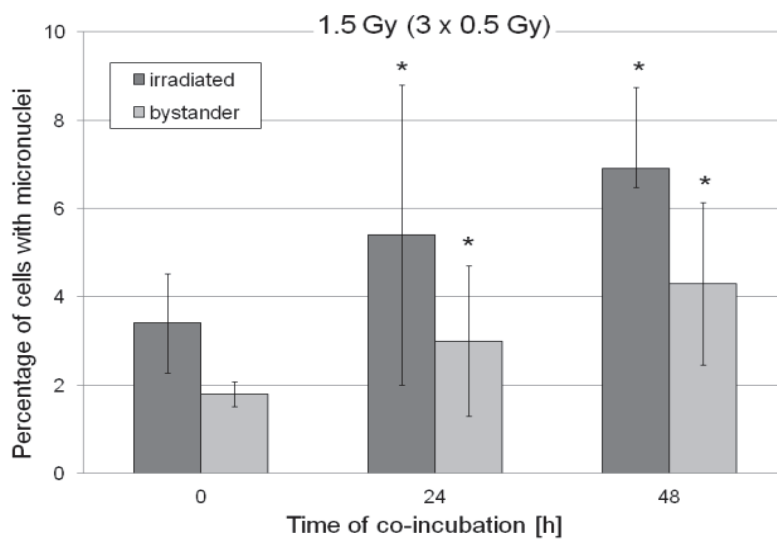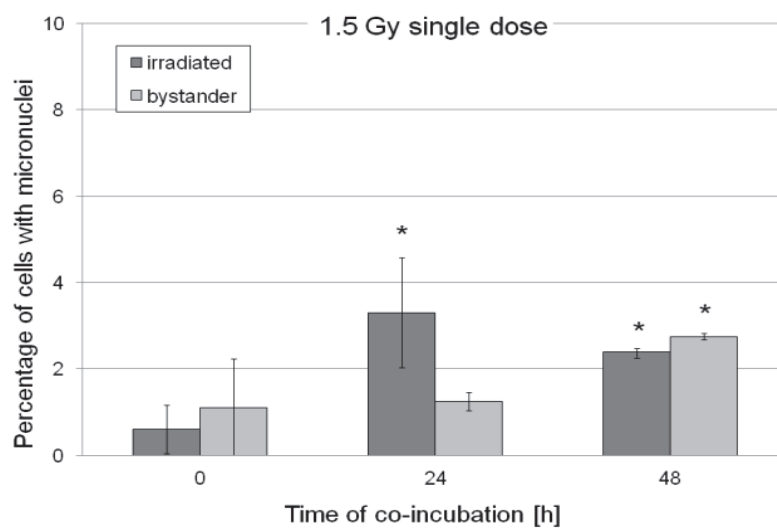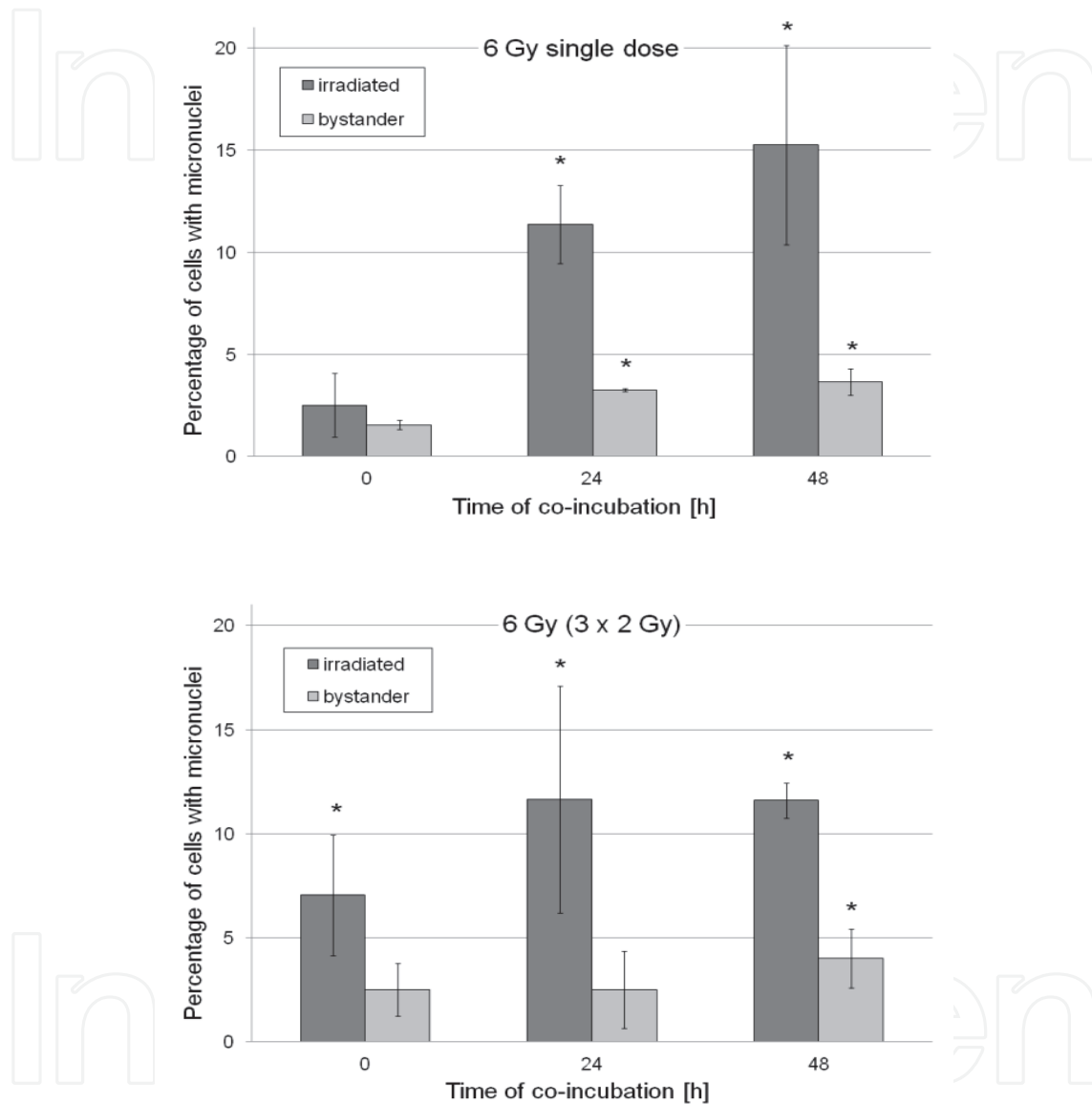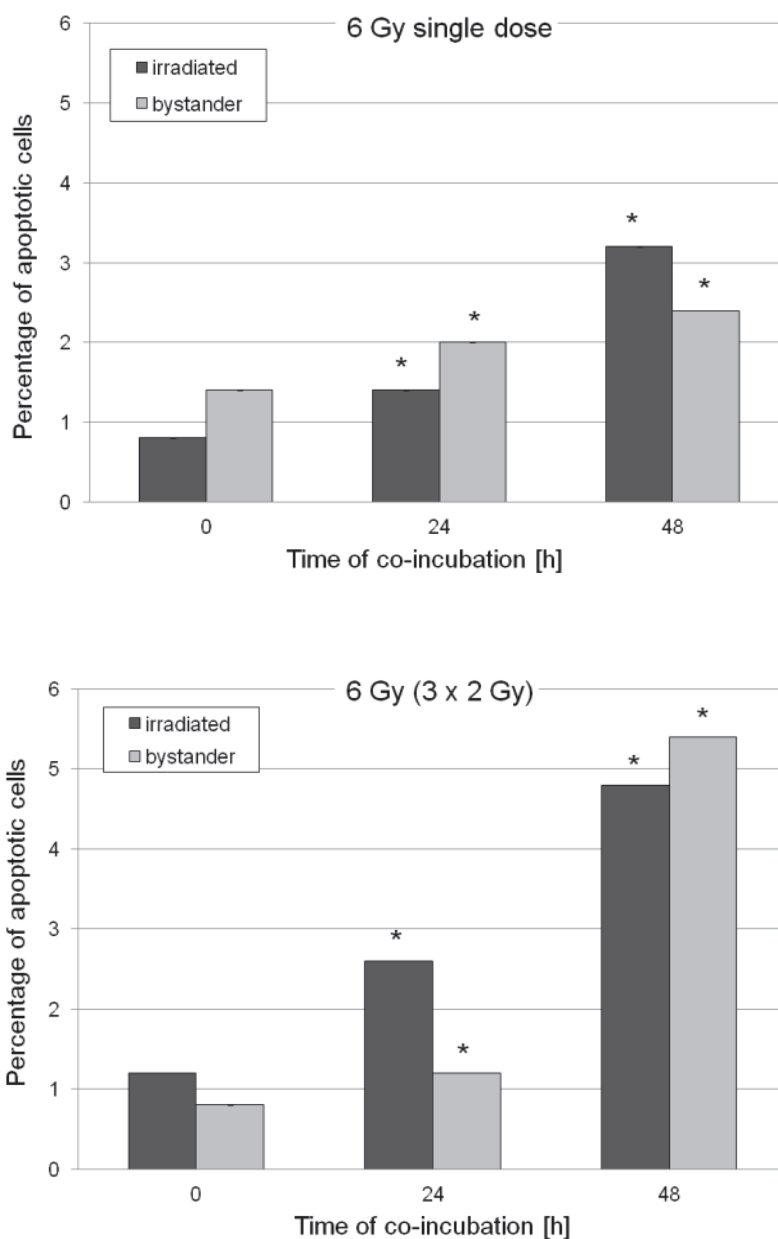
(* denotes statistical difference from corresponding control, p<0.05, Student's t-test).

Fig. 2. Yield of micronuclei induction in Me45 melanoma cells irradiated with single or fractionated doses, in comparison with bystander cells. Data show means ± standard deviation and were obtained from three independent experiments
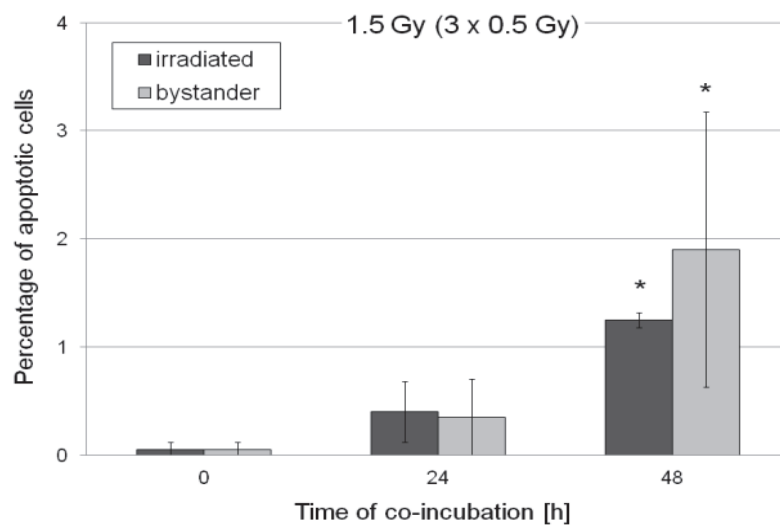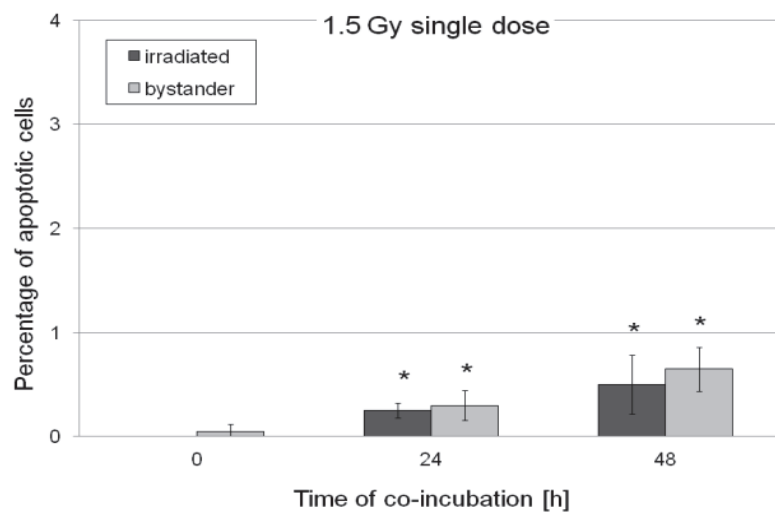
(* denotes statistical difference from corresponding control, p<0.05, Student's t-test).

Fig. 3. Yield of apoptosis induction in Me45 melanoma cells irradiated with single or fractionated doses, in comparison with bystander cells. Data show means ± standard deviation and were obtained from three independent experiments

Our data indicate that the bystander effect may play some role during fractionated radiotherapy and should be regarded as an important part of ionizing radiation effect on living cells. Although fractionated irradiation was also applied *in vivo* to study the bystander effect on the level of DNA epigenetic changes in the non-exposed spleen of cranial irradiated mice (Ilnytskyy et al., 2009), the fraction doses used were far below those clinically applied. However, the authors observed that acute irradiation induced more pronounced bystander effect than fractionated irradiation.

8. Radionuclide induced bystander effect

Induction of the bystander effect is prevalent at low radiation doses and low dose rates (Seymour and Mothersill, 2000), the characteristic features of targeted radionuclide treatment of cancer. Thus, one could expect that bystander effects induced by targeted radionuclides could have a strong impact on radiotherapeutic and diagnostic treatment (Prise and O'Sullivan, 2009). An increasing body of data indicates the involvement of bystander phenomenon after radionuclide application under experimental conditions. It can appear as damaging or protective effects in dependence on dose and dose rate. A very low dose of photon radiation (~ 30 keV) emitted by iodine-125 radioisotope (4mGy dose/day to 1,4 mGy/day) during a three month exposure of hybrid HeLa cells with human fibroblasts caused resistance of these cells to neoplastic transformation when they were challenged by subsequent irradiation with 3 Gy of ^{137}Cs gamma rays (Elmore et al., 2008). Lowering of dose rate below 1mGy/day abolished the adaptive answer, suggesting that low dose-rate above a certain threshold is responsible for this type of radio-adaptation. The damaging bystander effect induced by radionuclide is also frequently observed in *in vitro* experiments. Various type of cells may differ however in response to radionuclide induced bystander signals. Chen et al. (2008) using ^{125}I seeds irradiated two lung cancer cell lines that had different sensitivities to HDR gamma-ray irradiation and investigated the bystander effect of DNA DSBs as histone H2AX phosphorylation, and micronuclei formation. They found that the proportion of bystander cells with micronuclei and number of γH2AX foci was higher in radiosensitive NCI-H446 cell line than in more radioresistant A549 cell line. Interesting from clinical point of view was the observation that bystander effect compensated for the nonuniform distribution of radiation dosage in their experimental system. However, radionuclide induced bystander effect depends on the linear energy transfer (LET) of radionuclide emitters, being either damaging, or protective (Boyd et al., 2006, Mairs et al., 2007). Cells exposed to media collected from gamma-irradiated cells exhibited a dose-dependent reduction in survival fraction at low dosage and a plateau in cell-kill at >2 Gy. Cells exposed to media from metaiodobenzylguanidine-treated cells ([^{131}I]MIBG, a low LET β-emitter), demonstrated a dose-response relationship with respect to clonogenic cell death and no annihilation of this effect at high radiopharmaceutical dosage. Contrarily, cells exposed to media from cultures treated with meta-[211]At-astatobenzylguanidine ([[211]At]MABG, a high LET α-emitter) exhibited dose-dependent toxicity at low dose, but elimination of cytotoxicity with increasing radiation dose, i.e. U-shaped survival curves (Mairs et al., 2007). Biologically similar analogs of halobenzylguanidines radiolabeled with radionuclides emitting β-particles (^{131}I-MIBG), α-particles ([211]At-MABG), or Auger electrons emitting ^{123}I-MIBG, were also tested in experiments performed by the same group (Boyd et al., 2006) on a human glioma cell line (UVW) and a cell line derived from human bladder transitional carcinoma (EJ138), both

transfected with neurotransmiter (NAT) gene that enabled greater MIBG uptake. A similar U-shaped bystander phenomenon was observed for clonogenic cell-survival curve in case of high-LET alpha and Auger-electron emitters. No corresponding plateau in toxicity was observed after exposure of cells to the medium from β-irradiated cells. The reason for such behavior is not clear as yet. However, identification of the pathways involved in this process might pinpoint ways of manipulating the bystander effect for therapeutic purposes, i.e. to gain selective increase in tumor cell killing, accompanied by reduced side effects in normal tissue. Proliferative bystander responses have been also observed *in vitro* after irradiation with β-particles emitted by tritiated thymidine (^{3}HTdR). The rat liver epithelial cells (WB-F344 line) not treated with tritiated thymidine (unlabeled cells), in the presence of radiolabeled cells that received absorbed doses from 0.14 – 1.7 Gy, showed statistically significant increase of cell growth by 9-10% in comparison to control (Gerashchenko and Howell, 2004). The mean energy of β-particles is only 5.7 keV, (a range of ca. 1 μm in water). Thus, the probability that β-particles emitted from radiolabeled cells will target the nucleus of adjacent unlabeled cells in non-confluent co-culture used in the study is very low, because the majority of unlabeled cells were far beyond the range of β-particles emitted from radiolabeled cells. The authors compared ^{3}HTdR (the β-emitter) results with their earlier results obtained for γ–rays and found that a much lower dose of radionuclide (0.14 Gy) induced maximum response of bystander cells, whereas the maximum bystander response to γ-rays was not seen, even up to 1 Gy. According to the authors it is possible that the differences in the bystander dose response between γ-rays and ^{3}HTdR may be related to higher relative biological effectiveness (RBE) that has been observed for ^{3}HTdR, as compared to γ-rays.

The presence of bystander effect initiated by *in vivo* decay of radionuclide was demonstrated by Xue et al. (2002). When human colon LS174T adenocarcinoma cells prelabeled with lethal dose of Auger electron–emitting 5-[^{125}I]iodo-2-deoxyuridine (^{125}IUdR) were subcutaneously co-injected with LS174T unlabeled cells into nude mice, a considerable inhibition of tumor growth was observed. Since the ^{125}I present within the cells is DNA-bound, and 99% of the electrons emitted by the decaying ^{125}I atoms have a subcellular range (<0.5 μm), and since the overall radiation dose deposited by radiolabeled cells in the unlabeled cells within the growing tumor is less than 10 cGy, these authors concluded that the results obtained are a consequence of a bystander effect generated *in vivo* by factor(s) present within and/or released from the ^{125}IUdR-labeled cells. Radionuclides differ in their physical characteristics such as type of decay, the mean energy, the half-life and range of penetration. However, in spite of the identical decay, the Auger electrons for both, ^{123}I (half–life, 13.3 h) and ^{125}I (half-life, 60.5 d) they differ in mean energy which is 1.234 MeV and 179 keV for ^{123}I and ^{125}I respectively (Prise, 2008). The bystander effect induced *in vivo* by co-injection of radiolabeled and unlabeled LS174T cells was totally different (Kishikawa et al., 2006). ^{125}I labeled cells stimulated tumor growth, and inversely, ^{123}I labeled cells inhibited tumor growth after subcutaneous co-injection of cell mixture into nude mice. Similar pattern of response was observed in experiment *in vitro*. These contrasting effects were accompanied by different biochemical events; supernatants from cultures with ^{125}I-labeled cells were positive for tissue inhibitors of metalloproteinases (TIMP1 and TIMP2), and those from cultures with ^{123}I-labeled cells were positive for angiogenin (Kishikawa et al., 2006). These all studies demonstrate the potential of internalized radionuclides to generate bystander effects *in vivo* for therapeutic treatment, however many question remain in regard to bystander signaling evoked by application of different radionuclide, as pointed out in the

review of Sgouros et al. (2007). E.g. are the protective or damaging effects specific for different types of radionuclides or type of cell? Is the in vivo bystander effect restricted to the damage to DNA by ionization secondary to Auger-elctron cascade or is it also possible when radionuclides deposit their energies within the cell cytoplasm or membrane? Additional studies are required to fully understand the bystander effects in radionuclide therapy.

9. *In vivo* bystander effect

Bystander effect in tissues distant from the radiation field, named "abscopal effect", was observed more than 50 years ago as haematological changes of bone marrow in children, who were given radiotherapy to the spleen in the treatment of leukemia (Parsons et al., 1954). Until recently, the abscopal effect was referred to the distant effects seen after local radiation therapy. Although the abscopal effect is potentially important for tumor control, it is still extremely controversial. However, it inspired *in vitro* and *in vivo* studies. It is believed mediated through cytokines and/or the immune system and results from loss of growth stimulatory or immunosuppressive factors from the tumor (Kaminski et al., 2005). The observation that irradiation of a murine tumor caused growth inhibition of another tumor outside of the radiation field was explained as the effect of immune system activation (Demaria et al., 2004). Interestingly, growth inhibition of tumors remote from the radiation field was tumor-specific. Camphausen et al. (2003) observed an abscopal effect as significant growth delay of distally implanted Lewis lung carcinoma and T241 fibrosarcoma cells in mice when they irradiated the non-tumorbearing legs. Furthermore, the authors compared this effect after fractionated irradiation with five 10-Gy fractions or twelve 2-Gy fraction and found dose dependent inhibition of tumor growth, being greater with higher fraction dose. Persuasive evidence of the bystander effect presence *in vivo* comes from experiments on rats in which the bases of the lungs were exposed to 10 Gy, while the remaining 70% of lungs were protected (Khan et al., 2003). A considerable increase in the DNA damage (micronuclei) was observed in the shielded lung. In addition, various parts of the lungs differed in the micronuclei frequency in response to direct irradiation, or only to bystander signals. The protective effects of two radical scavengers, superoxide dismutase (SOD) and nitro-L-arginine methyl ester (L-NAME), suggest that inflammatory cytokines induced by the irradiation may be involved in the initiation of a reaction generating reactive oxyradicals and nitric oxide that cause indirect DNA damage, both in and out of the radiation field (Khan et al, 2004). The mediators of bystander effect *in vivo* may be macrophages and inflammatory cytokines. Calveley et al. (2005) showed that activation of macrophages and expression of inflammatory cytokines fluctuated in a cyclic pattern in the directly irradiated and bystander regions of the same lung tissues. Cytokines including IL-1a, IL-1 IL-6, TNF-a and TGF-β were expressed to a similar degree in both, radiation targeted and non targeted lung tissues when measured on RNA levels. The results of animal studies involving irradiation of one side of the mouse body with 1 Gy X-ray showed DNA DSBs induction and increase in the levels of Rad51 (DSBs repairing protein) in non exposed skin (completely protected by lead shield). Furthermore, the levels of two methyl-binding proteins known to be involved in transcriptional silencing, MeCP2 and MBD2, were also increased in bystander tissue suggesting that radiation induced bystander effect may be epigenetically regulated. Global DNA hypomethylation is a typical feature of cancer cells. The methylation is one of the many types of histone modification processes which include, phosphorylation,

acetylation, and ubiquitination, referred to epigenetic changes. Pogribny et al. (2004, 2005) investigated the effect of low-dose radiation exposure on the accumulation of DNA lesions and alterations of DNA methylation and histone H4-Lys20 trimethylation in the thymus tissue using an *in vivo* murine model. They found that fractionated whole-body application of 0.5 Gy X-ray leads to decrease in histone methylation and DNA damage accumulation in the thymus gland. The radiation-induced global genome DNA methylation changes were shown to be dose-dependent, sex- and tissue specific and long-persistant. Tissue specificity of bystander responses within the same organism has also been examined by Ilnytskyy et al. (2009). They analyzed changes in global DNA methylation in spleen of mice whole-body or cranial exposed to single 0.5 Gy of X-rays or to the same dose given in five 0.1 Gy fractions. After acute cranial exposure the major changes were observed in the animal spleen such as a significant loss of global DNA methylation 6 hr, 96 hr, and 14 days after irradiation, resembling those induced in whole body irradiated rats. These changes also include DNA binding protein methylation, expression of methylotransferases and the methyl group binding retrotransposomal element LINE-1, and overexpression of micro RNA, miR-194. Therefore, these transcriptionally regulated epigenetic changes seem undoubtedly to be related to the radiation induced bystander effect, although they may be specific to certain tissues, because similar changes were absent in the dermal tissue (Koturbash et al, 2007, Ilnytskyy et al., 2009). *TP53* overexpression, change of proliferation rate measured as Ki67 antigen expression, as well as the increase in the percentage of apoptosis and DNA double strand breaks, the marker of which was the histone H2AX phosphorylation were also observed in bystander spleen of mice exposed to 1 Gy X-rays to their heads. These changes persisted from 24 hours to seven months (Koturbash et al, 2008). All of those experiments indicate that cells and tissues irradiated *in vivo* send signals which are transmitted by paracrine and endocrine systems and are able to induce damage in DNA, apoptosis, clastogenic effects, and epigenetic changes that lead to genetic instability. The consequence of the long-persisting changes may be the late effects including mutation and induction of second primary cancer. In good agreement with data presented above are results of the elegant study on bystander effect in human tissue models, which preserve the three-dimensional structure and communication of cells present in tissues *in vivo* (Sedelnikova et al. 2007). The artificial skin which is able to survive 2-3 weeks in culture was irradiated with microbeam helium ions (7 MeV ⁴He, range in tissue 31 µm). The beam size was restricted to a 1 to 2 nuclei width along the line of irradiation. Bystander effect was studied on histological slides prepared at various time post irradiation (up to seven days). The authors observed increases in bystander cells the double strand breaks formation, followed by increased levels of apoptosis and micronucleus frequency, hypomethylation of nuclear DNA, and by an increased fraction of senescent cells. These findings point out the DNA DSBs induced by bystander signals as precursors of different cellular consequences in human tissues.

10. The potential clinical consequences of radiation induced bystander effect

Although direct extrapolation of data from *in vitro* experiments to *in vivo* radiotherapy is not possible, (three-dimensional structure of tissues), one could assume that the bystander effect implies a risk of post-radiation complications in healthy tissues. It is suggested that genetic instability, which takes the form of delayed reproductive death (DRD), can participate in late side effects in patients treated with radiotherapy, because of damage, increased cell loss

and longer recovery (Hendry, 2001). Increased level of chromosome aberrations and micronuclei was detected in the head and neck cancer patients undergoing radiotherapy within a year post treatment (Gamulin et al., 2008). DRD phenomenon associated with the presence of an increased percentage of stable and unstable chromosome aberrations in lymphocytes was detected in patients irradiated because of ankylosing spondylitis even several years after radiotherapy. Furthermore, increased mortality was related to single treatment course of X-rays because of this diseases (Smith & Doll, 1982). However, other studies performed in adults many years after radiotherapy in childhood haven't shown genetic instability (Tawn et al., 2005). Neither was it shown in persons having professional contact with radiation, who have suffered internal plutonium contamination at least 10 years previous to the study (Whitehouse and Tawn, 2001). Furthermore, creation of mutator phenotype as a result of genetic instability seems to increase the probability of induction of tumors. It has been shown on an animal model that ionizing radiation induces genetic instability emerging as delayed *TP53* mutations and more frequent transformation of mammary gland epithelial cells, leading to the development of cancer (Ulrich and Ponnaiya, 1998). Compared to healthy persons, irradiated cancer patients show increased incidence of second-wave primary cancers (Boice et al., 1985, Brenner et al., 2000), although the bystander effect does not need to be the only cause of such events. It is well-known that genetic predispositions and environmental factors may have significant influence on the formation of tumors (Mohandas, 2001).

Together with modern techniques of irradiation, such as three-dimensional conformal radiation therapy (3D-CRT) or intensity-modulated radiation therapy (IMRT), the purpose of which is to reduce radiation dose delivered to healthy tissues, there is an increased risk of adverse effects resulting from a possible bystander effect, especially because in these techniques larger volumes of normal tissues are exposed to a small dose (Hall, 2006). The risk of secondary cancers is increased especially in prostate cancer (Brenner et al., 2000) and cervical cancer (Boice et al., 1985, Kleinerman et al., 1995, Chaturvedi et al., 2008, Trott, 2009). Prostate surgery and radiotherapy are methods having comparable efficacy, therefore any late consequences in the form of secondary tumors should be taken into account, especially in younger people with a perspective of long-time survival. Brenner et al. (2000) compared the incidence of second-wave primary cancers in prostate cancer patients treated with surgery only (more than 50 000) to that in patients treated by radiotherapy (more than 70 000) and observed a statistically significant, although small, increase in the risk of secondary cancers in the latter group (6%, p = 0.02). This risk was associated with dose and latent time and grew with increasing survival time, amounting to 15% for patients surviving over 5 years and to 34% for those surviving over 10 years. The emerging cancers were solid tumors, such as bladder, bowel and lung carcinomas and sarcomas, the latter within the field of irradiation. The authors did not observe leukemia cases. The risk of secondary cancers after radiotherapy of cervical cancer is comparable to that of prostate cancer. Kleinerman et al. (1995) compared the risk of secondary cancers in radiation-treated, invasive cervical cancer patients (almost 50 000) with that in a group of non-irradiated patients surviving more than 30 years and showed a 12% increase in newly-diagnosed secondary cancers, where the increase was 15% after 10 years and 26% after 20 years post radiotherapy. Cancers of colon, bladder, rectum, vagina and ovary were within the fields covered by the high-dose radiation, but there were also few cases of leukemia. However, half of secondary neoplasms accounted for lung cancer. Occurrence of cancer of the lung, the organ relatively distant from the original tumor irradiation field, in which the radiation

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dose was estimated at ca. 0.6 Gy (Brenner et al., 2000), appears to have been associated with the bystander effect induced by signaling molecules in the neighborhood, and with potentially mutagenic carcinogens generated by irradiated cells, although environmental factors, genetic background and patients' lifestyle could also significantly contribute.

Calculations of the equivalent whole-body dose in the case of high-energy IMRT irradiation technique (Followill et al., 1997), indicate that, in comparison with conventional radiotherapy, the risk of secondary solid cancers has increased considerably. This increase is dependent on the X-ray energy and is 1% for 6 MV, 4.5% for 18 MV and 8.4% for 25 MV compared with 0.4, 1.6 and 3%, respectively, for those same radiation energy of X-rays given in a conventional way. Furthermore, as a conclusion from this study it appears that the risk of leukemia also increases after IMTR technique. The question of secondary tumors, as a succession of radiotherapy, was investigated in several recent studies [Suit et al., 2007, Trott K-R., 2009, Tubiana M., 2009, Xu et al., 2008]. Based on epidemiological and experimental radiobiological data, Suit et al. (2007) concluded that the relationship of tumor induction risk and dose is complex and differs not only between species of animals, between individuals of the species concerned, but it may also be different for various tissues and organs. Specifically, the risk increases with dose in the 1-45 Gy range for gastric and pancreatic cancer, but is stable in the 1-60 Gy dose range for bladder cancer, and even negative for colon cancer. These phenomena are difficult to explain. They could more likely be the result of genetic instability than the effect of bystander signals at lower doses, as well as result from inhibition of signals originating from cells lethally damaged by higher doses.

It seems that bystander effect can have beneficial consequences, particularly in radionuclide therapy as described above and probably in brachytherapy (Brans et al., 2006) in which tumor cells irradiated by intake or absorption of isotope energy are in the immediate vicinity of non-irradiated cells inducing in them the effect. The bystander effect can also increase damage to cancer cells during treatment with boron neutron capture therapy (BNCT) (Barth et al., 2005). As previously described, the "abscopal effect" is also an example of manifestation of the beneficial effects of irradiated cells, even at a distance from their location [Kaminski et al., 2005]. However, it is also possible that pro-survival signals, sent by lethally damaged cells, may increase the chances of survival of other, less damaged tumor cells within the field of irradiation and may pose a risk of local recurrence (Mackonis et al. (2007). The mutual communication between normal and cancer cells leading to radioprotective effect to radiation targeted cancer cells, as presented above, can also be taken into consideration. Furthermore, one can expect that the individuals exposed internally to radionuclides for routine diagnostic nuclear medical procedures might be at risk of bystander effect however, prediction whether it will be damaging or protective requires further studies.

11. Conclusion

Radiation induced bystander effect (RIBE) is unquestionable biological phenomenon which elicits in cells not directly irradiated but being in the neighborhood of targeted cells, or being exposed to molecular signals disclosed by irradiated ones. It has been found in variable *in vitro* and *in vivo* systems. RIBE predominate at externally applied low doses and low dose-rate, although many data confirm its presence at clinically used doses and radionuclide exposure. It may be either detrimental or potentially beneficial event depending on dose, dose-rate, means of irradiation, cell types and environmental conditions pointing out to its

very composed nature. The bystander effect induced by radionuclide intake seems to be the most susceptible to modulation of bystander signaling for clinical purposes aimed to improvement of the therapeutic ratio. However, the potential and real clinical consequences of bystander effect are, as yet, not predictable. We are not able to predict whether and what form, damaging or radioprotective, will the bystander effect take in the patient without knowledge of patients/tumor response to low or high dose-rate irradiation, tumor vasculature and normal cells infiltration. The more, we are not yet able to modulate the response of the patient to the signals generated in the process. Therefore, additional studies are required to address these questions.

12. Acknowledgment

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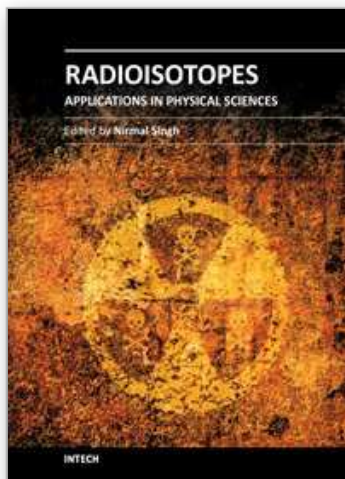
Radioisotopes - Applications in Physical Sciences

Edited by Prof. Nirmal Singh

The book Radioisotopes - Applications in Physical Sciences is divided into three sections namely: Radioisotopes and Some Physical Aspects, Radioisotopes in Environment and Radioisotopes in Power System Space Applications. Section I contains nine chapters on radioisotopes and production and their various applications in some physical and chemical processes. In Section II, ten chapters on the applications of radioisotopes in environment have been added. The interesting articles related to soil, water, environmental dosimetry/tracer and composition analyzer etc. are worth reading. Section III has three chapters on the use of radioisotopes in power systems which generate electrical power by converting heat released from the nuclear decay of radioactive isotopes. The system has to be flown in space for space exploration and radioisotopes can be a good alternative for heat-to-electrical energy conversion. The reader will very much benefit from the chapters presented in this section.

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