CELLULAR BYSTANDER EFFECTS AND RADIATION HORMESIS

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Abstract. Bystander effects describe the effects of extracellular mediators from irradiated cells on neighbouring non-irradiated cells resulting in radiation-induced effects in unirradiated cells. Although the underlying mechanisms are largely unknown, it is widely recognised that two types of cellular communication (i.e. via gap junctions and/or release of molecular messengers into the extracellular environment) play an essential role. Additionally, the effects can be significantly modulated by parameters such as cell type, cell-cycle stage and cell density. Some of the common bystander effects or biological end points which are evidenced after low-dose irradiation are: chromosomal instability, cell killing and delayed cell death, mutagenesis, micronucleus formation, gene and protein expression changes. Through these end points it is likely that bystander effects can be both detrimental and beneficial. By increasing mutation levels of cells bystander effects increase the likelihood of genetic defects and in turn cancer. On the other hand by removing damaged cells from the population and preventing the growth of cancer cells, bystander effects are beneficial.

Radiation hormesis is a term used to relate the beneficial effects of small doses of radiation on living cells, whether plant, animal or human. Experiments on bacteria, plants and animals have demonstrated that several biological mechanisms are stimulated by low dose radiation, such as: protein synthesis, gene activation, detoxication of free radicals and stimulation of the immune system. These mechanisms were also observed in humans.

The present review paper is a compilation of the most recent data on bystander effects and the possible implications of cellular response to radiation on cell growth and development.

Keywords: adaptive response, bystander effect, hormesis, low dose radiation

INTRODUCTION

It is now known that biological effects of low-dose radiation are far more complex than predicted by the linear no-threshold model. The adaptive-response model and the bystander effect clearly illustrate this complexity. The adaptive-response model, postulates that certain doses of low-dose radiation may be beneficial. Usually the adaptive response is induced with 1–100 mGy of γ-rays. It is important to note that a 1 mGy dose of γ-rays generates on average one track of clustered reactive oxygen species per nucleus and is therefore considered the lowest dose that can affect a whole cell culture or animal [16]. With 1 mGy, approximately 3% of irradiated cells undergo a DNA double strand break. This model was first proposed in 1984 to explain the finding that cultures of human lymphocytes growing in low concentrations of radioactive thymidine developed fewer chromosomal aberrations than cultures of non-radioactive lymphocytes when both were challenged with high-dose radiation [14]. On the other hand, the bystander-effect model, postulates that low-dose radiation may be even more damaging than that predicted by the linear no-threshold model. It has been reported that 1% of cells in cell cultures directly irradiated with an α-particle, "transmitted" the chromosomal damage to 30% of the total cell population [12], via cell-to-cell communication.

Radiation-induced bystander effects are a subject of great interest both in radiation protection and, lately, in radiotherapy. It was concluded that bystander effects (radiation-induced effects detected in non-irradiated cells) predominate at low doses. The possible impact of bystander effects in radiotherapy has become a hotly debated topic, since normal tissue effects might be more pronounced than previously thought.

In contrast, studies have shown that low doses of radiation can induce an adaptive response in the exposed cells, making them resistant to subsequent doses. There is, therefore, evidence of conflicting phenomena at low doses: bystander effects which exaggerate the effect of radiation at low doses and adaptive response which confers resistance to subsequent fractions of radiation.

CELL AND RADIATION

Although ionizing radiation can produce a broad spectrum of DNA lesions including damage to nucleotide bases, unrepaired or misrepaired DNA double-strand breaks (DSB) are thought to be the principal lesions responsible for the induction of genetic changes in mammalian cells, including chromosomal abnormalities and gene mutations.

Cells possess a complex set of signalling pathways for recognizing DNA damage and initiating its repair. The ATM gene is one of the main sensors of DNA damage, which activates by phosphorylation a variety of proteins involved in cell cycle control and DNA repair. Unrepaired or misrepaired DSB lead primarily to large-scale genetic changes, which are frequently manifested by chromosomal aberrations. The pathway used to repair DNA damage depends on the stage of the cell cycle.

The double-helical structure of DNA is ideally suited for repair because it carries two separate copies of all the genetic information - one in each of its two strands. Thus, when one strand is damaged, the complementary strand retains an intact copy of the same information, and this copy is generally used to restore the correct nucleotide sequences to the damaged strand.

Some of the major DNA repair pathways involved in the cellular response to ionizing radiation are the

following: base excision repair, nucleotide excision repair, non-homologous end joining, homologous recombination repair and mismatch repair.

The damage produced in a mammalian cell nucleus by 1 Gy of low LET radiation consists of approximately 1000 DNA single-strand breaks and 40 double-strand breaks (1 DSB per chromosome per Gy). It was shown by track structure analysis that 50% of DSB will have other types of DNA damage in close proximity (clustered damage). A double-strand break is believed to be the most important radiation-produced lesion in chromosomes since the interaction of two double-strand breaks may result in cell killing, mutation, or carcinogenesis.

DETRIMENT

Detriment is a measure of the total harm that will result after exposure to radiation. Generally, the detriment from radiation is considered to be caused by stochastic effects, since for deterministic effects the equivalent doses are below the threshold.

There are three possible models investigating the relationship between detriment and dose: the linear no-threshold, the threshold and the hormetic models (Fig. 1).

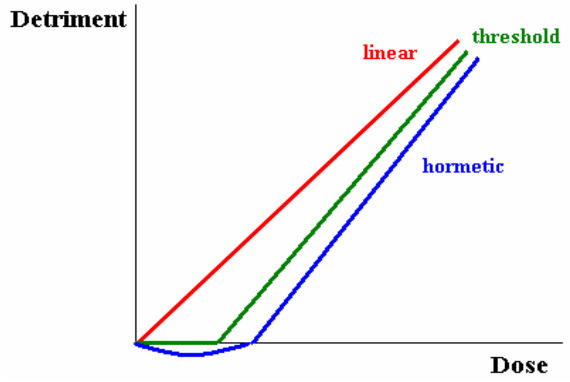

Figure 1. Detriment as a function of dose

The linear, no-threshold (LNT) theory of radiation was originated in the 1950s as a prudent operational guideline. In some opinions, the LNT theory was never more than a working hypothesis, which has acquired the appearance of fact even though no one has ever generated any evidence for it. The theory has been derived from direct extrapolation of the harm at high exposures of radiation to the very small doses to which all living cells are exposed to.

The principal basis for the LNT is theoretical, and very simple. A single particle of radiation hitting a single DNA molecule in a single cell nucleus of a human body can initiate a cancer. The probability of a cancer initiation is therefore proportional to the number of such hits, which is proportional to the number of particles of radiation, therefore to the dose.

The problem with this simple argument is that factors other than initiating events affect the cancer risk. Our bodies have biological defence mechanisms which prevent the vast majority of initiating events from developing into a fatal cancer. For instance:

- Our bodies produce DNA repair enzymes, which repair the effects of initiating events with high efficiency.
- Cancer development is a multi-stage process, and consideration must be given to how radiation may affect stages other than initiation.
- Radiation can alter cell-cycle timing, which can affect cancer development. Damage repair is effective only until the next mitosis, so changing this available time can be important.
- There is fine evidence that the immune system plays an important role in preventing cancer development, and its potency can be altered by radiation.

A completely opposing theory to the linear no-threshold one is radiation hormesis. There are now substantial cellular and molecular investigations indicating that low-level exposures to radiation can cause adaptive responses, therefore enabling protection from subsequent irradiation. It was indicated that such defence mechanisms prevent and repair DNA damage, and reduce the number of surviving mutations.

RADIATION HORMESIS

The “no threshold” hypothesis of the radiation effects on cells is a very controversial one since an obvious conclusion resulting from this theory is that even the natural background radiation is harmful. For estimating risk factors for radiation carcinogenesis, epidemiological studies of about 90,000 survivors of the nuclear attack in Japan have been made [5]. The studies indicated the cancers are induced by doses, thousands of times higher than the annual dose from background radiation.

After the acceptance of a threshold dose for biological damage, studies have been undertaken for doses below the threshold value (very low dose radiation). While few years ago the low dose radiation was considered harmful, now the other side of the coin is supposed to be true: very low dose radiation has beneficial effects. The positive effect of radiation on humans is called radiation hormesis. The word "hormesis" is derived from the Greek word "hormaein" which means "to excite" and it has been founded by Southam and Erlich in 1943. Hormesis is an effect where an agent given in a small dose acts like a stimulant but it is an inhibitor in large doses (examples of such agents: medication, alcohol, radiation from the Sun). In other words, low doses of various agents evoke a biopositive effect while large doses of the same agents produce a bionegative effect.

The first complete report on radiation hormesis has been published in 1980 by Luckey. His work consists of an ample review of pro-hormesis reports [8]. His message through the publication is that “small and large doses induce opposite physiologic results”.

The main concern in radiation protection at molecular level is protection of the DNA. It is a well-known fact that high doses of radiation are damaging to the DNA suppressing also the DNA-damage-control biosystem. It was shown that low dose radiation is able to stimulate and improve the DNA damage-control

which results in a reduced number of misrepair and unrepair within the double helix [15]. Several experiments have been conducted with invertebrates kept in radiation-deficient conditions. The general result was that their optimal development was unachieved, therefore the conclusion that small doses of radiation are vital for good health. Many biological mechanisms are stimulated by low dose radiation: protein synthesis, gene activation, detoxication of free radicals, stimulation of the immune system, etc. These mechanisms were observed in bacteria, plants, animals and also in humans (table 1).

Table 1. Biological mechanisms stimulated by low dose radiation in plants and animals

Receptor	Biological effect
Bacteria Plants Animals	stimulation of protein synthesis
	gene activation
	detoxication of free radicals
	stimulation of the immune system
Animals Plants	increased growth
	increased fertility
	increased longevity
	reduction in cancer frequency

Dealing with hormesis there is no clear demarcation between harmful and harmless dose. Although the concept of radiation hormesis is usually applied to physiological benefits from low LET radiation in the range of 1-50 cGy, some other publications consider the level of background radiation as low dose. Whatever the exact dose range, there is a common agreement regarding the hormetic outcomes of low level exposure [17]: increased longevity, increased growth and fertility of both plants and animals and reduction in cancer frequency.

Even if hormesis, as a concept, was not recognized in unanimity and there are many controversial theories regarding the biopositive effect of low dose radiation, there is a strong logical support behind it: hormesis has an evolutionary basis. The actual radiation background is lower than hundreds of thousands years ago when many species of plants and animals which disappeared along the centuries have grown and thrived in those conditions and many of them were more developed than the common ones nowadays. Since background radiation was always present and it is part of our environment, each organism became adapted to these conditions so humankind is living with radiation.

BYSTANDER EFFECTS

Bystander effects are radiation-induced effects observed in non-irradiated cells in the proximity of irradiated cells (Figure 2a).

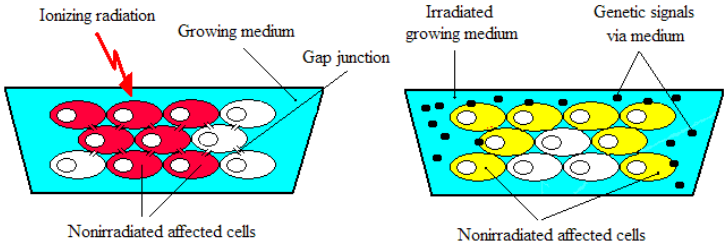

Figure 2. Illustration of the bystander effect by **a)** cell-to-cell communication via postulated gap junctions; **b)** via signals from medium transferred from irradiated cells.

The most common experimental method to demonstrate the viability of bystander effects is the microbeam experiment ('single-cell microbeam') in which cells on a Petri dish are individually irradiated by a predefined number of α particles, allowing an individual assessment of the cells. Typically, the experiment shows a larger number of cells with radiation-induced damage than the cells traversed by the α particles. Several experimental groups have developed single-cell microbeams to evidence cellular changes through bystander effects [2, 3, 13]

Another method substantiating the existence of radiation-induced bystander effects is the use of growth medium harvested from irradiated cells (from exposed Petri dishes or flasks) on non-irradiated cells (figure 2b). There is evidence for release of a survival controlling signal into the medium during irradiation [10], suggesting that cell-to-cell contact is not necessary to induce bystander effect when irradiated medium is transferred to the non-irradiated cells.

Therefore, bystander effects can be identified through:

- direct methods (direct cell-to-cell contact), when cells are irradiated and the biological effect of radiation observed in the neighbouring cells;
- indirect methods (irradiated cells are not in contact with the non-irradiated cells), when the growth medium is transferred from irradiated cells to non-irradiated cells and the biological effects observed on the non-irradiated cell population.

There is some indication that cell-to-cell communication via gap junctions may be more common for signals induced by high LET radiation while low LET radiation induces bystander signals that are transferred to unexposed cells through medium. However the evidence for a clear-cut difference is not strong [7].

Possible mechanisms proposed to be involved in this phenomenon include endocytosis of toxic cell debris, apoptosis, exposure to soluble toxins, involvement of undefined immune responses, or blood vessel destruction [9].

In the 60's Subak-Sharpe [20] postulated that "a cell in contact with a different cell may be capable – at least

for some time – of a metabolic function for which it lacks genetic information. Thus, in a monolayer, an individual cell's metabolic capability would not be totally limited by the cell's own genotype, but rather by the total gene pool of all the cells with which it is directly or indirectly in contact". It has now been scientifically proven [6, 21] that the damage to the non-irradiated cells is transmitted by intercellular signals through gap junctions, which are small channels that are formed between neighbouring cells allow to sharing of small molecules and ions. It has been reported that the bystander effect is of a much larger magnitude when cells are in such close proximity to allow gap junction communication, than that able to be demonstrated in medium transfer experiments [20].

The exact type of the signals involved in the bystander effect is still under investigation. However, there is some evidence that oxidative stress may play a role in the damage inflicted to neighbouring cells. The evidence is based on the observation that reactive oxygen species are highly cytotoxic and could theoretically pass through the gap junctions directly, or their production induced by other signalling molecules. The notion that hypoxic cells present with reduced, or even absent bystander effects is therefore possible. It is also likely that normal, well oxygenated cells experience more pronounced bystander effects than hypoxic cells.

Some of the common bystander effects or biological end points which are evidenced after both low-dose and high-dose (radiotherapy) irradiation are: chromosomal instability, cell killing and delayed cell death, mutagenesis, micronucleus formation, gene and protein expression changes. Through these end points it is likely that bystander effects can be both detrimental and beneficial. By increasing mutation levels of cells bystander effects increase the likelihood of genetic defects and in turn cancer. On the other hand by removing damaged cells from the population and preventing the growth of cancer cells, bystander effects are beneficial. It is assumed that both beneficial and detrimental effects occur at the same time [22].

There is published evidence that bystander mechanisms following irradiation are possibly involved in adaptive responses. Published data for low dose, low LET radiation demonstrates that signals produced by irradiated cells can induce protection against higher doses of radiation [11]. Although cell death is a common response following exposure to bystander signals, this can be considered a protective response, as damaged cells will be stoped from undergoing further divisions which ultimately lead to mutagenesis and carcinogenesis. In experiments simulating fractionated treatment, the first dose of radiation has proven to be more effective than subsequent doses. This observation was equally valid whether the first radiation dose was directly targeting the cells or the dose has derived from bystander medium (medium harvested from previously irradiated cells and used on non-irradiated culture). These experiments [11] conflict with the still accepted ‘isoeffect per fraction’ theory, since cell kill varied significantly between the first and subsequent doses.

Experiments on mouse embryonic fibroblast cell line cultures (C3H 10T1/2) have been conducted to assess the relative importance of the adaptive response and the bystander effect induced by radiation [18-19]. Single-cell microbeam was delivered from 1 to 12 alpha particles through the nuclei of 10% of the cultured cells observing that more cells were inactivated than hit by alpha particles. It was noticed that the magnitude of this bystander effect increased with the number of particles per cell. To assess the extent of the adaptive response to radiation, the cells were irradiated beforehand with 2 cGy of γ rays, which has cancelled out around half of the bystander effect produced by the alpha particles. The results are in agreement with the data published by Azzam *et al.* [1] on pre-exposure of C3H 10T1/2 cells to low-dose γ radiation showing a reduction in oncogenic transformation frequency after exposure to a 4 Gy challenge dose.

The cellular adaptive response to ionizing radiation is manifested through: reduction in chromosome aberrations, reduction of mutation frequency and also reduction in micronucleus formation.

The two processes are conflicting in the sense that they operate in opposite directions:

– the bystander effect, enhances the effect of low doses, and
– the adaptive response, confers resistance to a subsequent dose.

Table 2. Consequences of bystander effect versus adaptive response

Bystander effect	Adaptive response
chromosomal instability	reduction in chromosome aberrations
cell killing/delayed cell death	
mutagenesis	reduction of mutation frequency
micronucleus formation	reduction in micronucleus formation
changes in gene and protein expression	

DISCUSSIONS

For over half a century it has been accepted that radiation-induced damage required radiation interaction with the cellular DNA. This interaction could occur by either direct ionization or by indirect ionization, via hydroxyl radicals produced in water molecules close to the DNA. However, over the past two decades, several experimental studies have shown that there is no need for interaction between cellular DNA and radiation in order to produce damage characteristic to ionizing radiation. The phenomenon by which unirradiated cells, in the vicinity of irradiated cells, present with radiation-specific injury is called the bystander effect.

The current review has presented two phenomena induced by low doses of radiation: the bystander effect and the adaptive response of cells. While the bystander effect can induce some biological endpoints (such as chromosomal instability, cell killing and delayed cell death, mutagenesis, micronucleus formation, gene and protein expression changes) which can be detrimental, these endpoints can be considered, at the same time, beneficial, through removal of damaged cells from the

population and prevention of cell growth of mutated origin.

It was also described that signals produced by irradiated cells can induce protection against a subsequent (and also higher) dose of ionizing radiation, conferring them adaptive response. The two mechanisms of bystander effects and adaptive response dominating at low doses are part of the cellular homeostatic response and there is little evidence that they translate into harm. This could, therefore, imply that low doses of radiation induce radiation hormesis.

REFERENCES

[1] Azzam, E.I., Raaphorst, G.P., Mitchel, R.E., (1994): Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells, Radiation Research 138: S28-S31.

[2] Folkard, M., Vojnovic, B., Prise, K.M., Bowey, A.G., Locke, R.J., Schettino, G., Michael, B.D., (1997): A charged-particle microbeam: I. Development of an experimental system for targeting cells individually with counted particles, International Journal of Radiation Biology 72: 375-385.

[3] Geard, C.R., Brenner, D.J., Randers-Pehrson, G., Marino, S.A., (1991): Single-particle irradiation of mammalian cells at the Radiological Research Accelerator Facility: Induction of chromosomal changes, Nuclear Instrumentation Methods B54: 411-416.

[4] Hall, E.J., (2003): The bystander effect, Health Physics 85: 31-35.

[5] Jaworowski, Z., (1997): Beneficial effects of radiation and regulatory policy, Australasian Physical Engineering and Sciences in Medicine 20: 125-138.

[6] Krutovskikh, V., Piccoli, C., Yamasaki, H., (2002): Gap junction intercellular communication propagates cell death in cancerous cells, Oncogene 21: 1989-1999.

[7] Lorimore, S.A., Wright, E.G., (2003): Radiation-induced genomic instability and bystander effects: related inflammatory-type responses to radiation-induced stress and injury? A review. International Journal of Radiation Research 79: 15-25.

[8] Luckey, T.D., (1980): Hormesis with ionizing radiation, CRC press, Boca Raton.

[9] Mesnil, M., Piccoli, C., Tiraby, G., Willecke, K., Yamasaki, H., (1996): Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins, Proceedings of the National Academic Sciences USA 93: 1831-1835.

[10] Mothersill, C., Seymour, C.B., (1998): Cell-cell contact during gamma irradiation is not required to induce a bystander effect in normal human keratinocytes: evidence for release during irradiation of a signal controlling survival into the medium, Radiation Research 149: 256-262.

[11] Mothersill, C., Seymour, C.B., (2002): Bystander and delayed effects after fractionated radiation exposure, Radiation Research 158: 626-633.

[12] Nagasawa, H., Little, J.B., (1992): Induction of sister chromatid exchanges by extremely low doses of alpha-particles, Cancer Research 52: 6394-6396.

[13] Nelson, J.M., Brooks, A.L., Metting, N.F., Khan, M., Buschborn, R.L., Duncan, A., Miick, R., Braby, L.A., (1996): Clastogenic effects of defined numbers of 3.2 MeV alpha particles on individual CHO-K1 cells, Radiation Research 145: 568-574.

[14] Olivieri, G., Bodycote, J., Wolff, S., (1984): Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, Science 223: 594-597.

[15] Pollycove, M., (1998): Nonlinearity of radiation health effects, Environmental Health Perspective 101: 363-368.

[16] Rothkamm, K., Lobrich, M., (2003): Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses, Proceedings of the National Academic Sciences USA, 100: 4973-4975.

[17] Sagan, L.A., (1987): What is hormesis and why haven't we heard about it before? Health Physics 52: 521-525.

[18] Sawant, S., Randers-Pehrson, G., Metting, N., Hall, E., (2001): Adaptive response and the bystander effect induced by radiation in C3H 10T1/2 cells in culture, Radiation Research 156: 177-180.

[19] Sawant, S., Randers-Pehrson, G., Geard C., Brenner, D., Hall, E., (2001): The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells *in vitro* can be initiated in the unirradiated neighbours of irradiated cells, Radiation Research 155: 397-401.

[20] Subak-Sharpe, J.E., Burk, R.R., Pitts, J.D. (1969): Metabolic co-operation between biochemically marked mammalian cells in tissue culture, Cell Science 4: 353-367.

[21] Trosko, J.E., Madhukar, B.V., Chang, C.C., (1993): Endogenous and exogenous modulation of gap junctional intercellular communication: toxicological and pharmacological implications, Life Science 53: 1-19.

[22] Waldren, C., (2003): Classical radiation biology dogma, bystander effects and paradigm shifts, Biological Effects of Low Levels Radiation 3: 1-40.