Cancer Letters 356 (2015) 137–144

Contents lists available at ScienceDirect

Cancer Letters

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

Mini-review

# Radiation-induced bystander effect: Early process and rapid assessment

Hongzhi Wang [a], K.N. Yu [b,c,a], Jue Hou [a], Qian Liu [a], Wei Han [a,*]

[a] Center of Medical Physics and Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, PR China
[b] Department of Physics and Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong
[c] State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong

ARTICLE INFO

*Article history:*
Received 14 June 2013
Received in revised form 9 September 2013
Accepted 26 September 2013

*Keywords:*
Radiation-induced bystander effect
Rapid assessment
Early process

ABSTRACT

Radiation-induced bystander effect (RIBE) is a biological process that has received attention over the past two decades. RIBE refers to a plethora of biological effects in non-irradiated cells, including induction of genetic damages, gene expression, cell transformation, proliferation and cell death, which are initiated by receiving bystander signals released from irradiated cells. RIBE brings potential hazards to normal tissues in radiotherapy, and imparts a higher risk from low-dose radiation than we previously thought. Detection with proteins related to DNA damage and repair, cell cycle control, proliferation, etc. have enabled rapid assessment of RIBE in a number of research systems such as cultured cells, three-dimensional tissue models and animal models. Accumulated experimental data have suggested that RIBE may be initiated rapidly within a time frame as short as several minutes after radiation. These have led to the requirement of techniques capable of rapidly assessing RIBE itself as well as assessing the early processes involved.



## 1. Introduction

Since the discovery of X-ray by Röntgen in 1895, ionizing radiation has been used in both diagnostic and therapeutic medical applications although its biological effects have not been fully understood. Extensive researches have been carried out on victims of atomic bomb explosions, occupational and accidental radiation exposure in the past century and have aroused the general awareness among general citizens on the potential benefits and risks of ionizing radiation [1]. Despite the vast benefits derived from various medical applications, radiation can be harmful and is well established as a carcinogen to living organisms [2].

Previous works in the past three decades have established that the main biological "target" of radiation was the cell nuclei, while DNA damages, or more precisely the subsequent genetic changes due to mis-repaired or un-repaired DNA damages, were deemed the most important biological effect. Presumably, no effects would occur in cells not traversed by radiation. This dogma has been widely adopted by the radiation protection agencies. However, this dogma has been challenged by scientific findings since 1990s, exemplified by the occurrence of genetic changes in non-irradiated cells in a partially irradiated cell population [3–7]. Such a non-targeted phenomenon, called radiation-induced bystander effect (RIBE), implies radiation risks to cells or tissues which have not been irradiated, e.g., when the body is only partially exposed to irradiation [8,9]. Subsequent investigations on RIBE employed various research strategies, including partial irradiation of cell populations, tissues or animal models with conventional broad-field or microbeam irradiation, medium transfer, co-culturing with irradiated cells in separate inserts, mixed co-culturing with irradiated cells, etc. [10–12]. As time goes by, RIBE has been reported to induce various biological effects including sister chromatid exchange (SCE), micronuclei (MN), DNA double strand breaks (DSBs), gene locus mutation, neoplastic, even tumor formation, etc. [11–13].

Studies on mechanisms underlying RIBE have identified possible signaling pathways. For example, irradiated cells would release RIBE signal(s) which "attacked" the neighboring or even distant cells either through cellular gap-junction intercellular communication (GJIC) or through diffusion in the medium [9,11,13]. Oxidative stress plays a very important role in the generation, release and propagation of these signal(s) [9,11,13]. The involved signaling pathways which might mediate RIBE transduction were reported in subsequent studies. The mitogen-activated protein kinases (MAPK) signal pathway, nuclear factor kappa B/prostaglandin-endoperoxide synthase (cyclooxygenase) 2 (NF-κB/COX-2) pathway, nitric oxide (NO) related signal pathway, inflammation-related signal pathways, etc., were found to be an integral part of RIBE transduction [9,12–14]. The nature of extracellular RIBE signaling molecule(s) has been explored in the past years. Accumulated evidences indicated that the molecules(s) being released by irradiated cells and acting as possible extracellular RIBE signals include NO, transforming growth factor beta 1 (TGF-β1), tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-8, etc. [9,12].

* Corresponding author. Address: 350 Shushanhu Road, Hefei 230031, Anhui, PR China. Tel.: +86 551 65595100; fax: +86 551 65591233.
*E-mail address:* hanw@hfcas.cn (W. Han).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.09.031

The ionizing radiations to which common people are exposed come from sources in our natural environment such as radon progeny, and from medical activities and some unpredicted accidents. The radiation dose involved in these conditions is mostly very low [15]. Even in the recent Fukushima-Daiichi nuclear power plant disaster (which occurred on 11 March, 2011) which was rated 7 on the International Nuclear Events Scale (INES) scale, medical checks harvested on 28 August, 2011 of 3514 workers who had worked at the plant since 11 March, 2011 showed that only 124 of them had received radiation doses above 100 mSv [16]. When compared to the direct effects of irradiation, RIBE is very weak in the medium to high dose range [1,17]. However, in the low-dose range, RIBE could not be ignored [10,15,17,18]. Recently, Yang et al. confirmed that RIBE dominated the response in the cell population irradiated with high-energy protons and iron ions at low doses (5 cGy) [19]. Therefore, when considering the low-dose radiation exposures relevant to public health, assessment of health risk caused by both the direct effect and RIBE would be indispensable, e.g., for relevant risk assessment or for planning the necessary medical treatments.

The presence of RIBE increased the health risk of low dose radiation exposure since more cells in addition to the directly irradiated cells were indirectly damaged by the radiation. During radiotherapy, RIBE would increase the possibility of genetic changes and even tumor induction in tissues beyond the "targeted" tumor. In the investigation of RIBE, some rapid assessment or testing methods have been established and these methods have helped radiobiologists understand the occurrence of RIBE within a short time frame (such as 1–2 h) post irradiation and to further explore the possible mechanisms of RIBE. These rapid assessment or testing methods will also help the health risk assessment after some unplanned exposure such as nuclear accidents or assault of "dirty bombs".

## 2. Rapid assessment of RIBE

RIBE had been assessed with SCE testing [3], MN testing [5], cell death or clonal survival assay, gene locus mutation [7,20–22], etc. For these biological endpoints, relative long time periods would be required, e.g., for the formation of chromosome aberration, cell apoptosis, cell clones and mutations. At least 1–2 days or even more than 1–2 weeks would be needed with these methods. Besides, extra biological factors might be involved in the relative long process of RIBE measurement, and these extra factors might make it more difficult to identify the mechanisms of RIBE. Fortunately, continued research studies on RIBE had established methods with assessment time frame of several hours. Through these techniques, radiobiologists had been able to detect the promptly recruited DSB damage sensors or repair factors, specific genes expression, cell cycle relevant proteins, or transcription factors in the bystander cells to assess their risk. Significantly, some research groups had been able to observe fast occurrence of RIBE in even less than 5 min after irradiation [23–25]. Apparently, knowing more details of the early process of RIBE will be very helpful in better understanding how the RIBE signals are generated and released, and how they modulate the bystander cells.

### 2.1. Phosphorylation of H2AX

DSB is the most important lesion among different DNA damages [26,27]. The research presented by Little's group showed higher yields of chromosome aberration (MN and SCE) [28,29] and mutation [30] in bystander cells deficient in DSB repair, when compared to those in wild-type cells and cells deficient in base excision repair. These results also insinuated that DSBs were induced in bystander cells as a result of RIBE.

Phosphorylation of the H2AX protein, one member of the histone families, on serine 139 (γ-H2AX) has been known as one of the early responses of cells to DSBs induced by various stimulating factors [27,31,32]. Sedelnikova et al. proved that each discrete γ-H2AX focus contained a single DNA DSB, and suggested that the assessment by counting the number of γ-H2AX foci in the cell nuclei was the most sensitive method to evaluate the DSBs in cells [33]. In 2005 three groups independently reported their researches about assessing RIBE with γ-H2AX immunofluorescence [34–36]. Using a co-cultured system with multi-well inserts, Yang et al. detected significant γ-H2AX formation in bystander human fibroblasts cultured in the inserts after X-ray irradiation [34]. In a full confluent human skin fibroblast population, Hu et al. detected more γ-H2AX positive cells than the number of cells hit by low-dose α particles, which was attributed to the existence of RIBE [36]. Sokolov et al. presented more definite proofs of RIBE-induced DSBs in cultured human fibroblasts with γ-H2AX immunofluorescence and the co-localization of γ-H2AX foci and other DSB-related factors, such as phosphorylated ataxia-telangiectasia mutated kinase (ATM), 53BP1 and components of the MRN complex after irradiation with prescribed numbers of α particles from a microbeam facility [35].

In the following years, more research groups used this technique to assess RIBE in their research models [23,37–41]. Yang et al. observed a two- to threefold increase in the number of γ-H2AX foci in bystander cells sharing the medium with cells, which had been irradiated with iron ions in a co-cultured system with multi-well inserts, as early as 1 h after irradiation and lasted at least 24 h [42]. The temporal change of γ-H2AX foci formation in the bystander cells, which constituted half of the fully confluent cell population and which were shielded from irradiation, showed distinct increase in the number of foci over the control as early as 10 min post irradiation and reached the maximum at 30 min [24,25]. In an artificial human tissue model, Sedelnikova et al. detected 4-6-fold increase in the number of γ-H2AX positive cells in the bystander cell population, as far as 2.5 mm away from the plane of irradiation precisely performed with a microbeam facility, and as early as 0.5 h post irradiation [43]. In *in vivo* animal models, RIBE-induced γ-H2AX formation and Rad 51 expression were detected in the lead-shielded bystander skin [44] or cerebellum [45] of mice at about 6 h after X-ray exposure. On one hand, the γ-H2AX immunofluorescence technique allowed rapid detection of RIBE-induced genetic damages. On the other hand, the correspondence between the numbers of γ-H2AX foci and DSB enabled detailed assessment on the extent of damages from RIBE.

### 2.2. 53BP1

The tumor suppressor p53-binding protein 1, known as 53BP1, is a protein that in humans encoded by the TP53BP1 gene, and 53BP1 is required for p53 accumulation and cell cycle checkpoint in response to DSB repair [46]. Sokolov et al. remarked that 53BP1 foci could be induced in bystander human skin fibroblasts, and these foci were co-localized with γ-H2AX foci in bystander cells, which were co-cultured with cells irradiated by α particles from a microbeam facility or which received the conditioned medium harvested from a γ-ray irradiated cell population [35]. Tartier et al. used 53BP1 foci to assess RIBE induced by precise irradiation on cell nucleus or cytoplasm with a microbeam facility, and their results showed that the fraction of 53BP1 positive bystander cells peaked at 1 or 3 h when the nucleus or cytoplasm were irradiated, respectively [47].

With this method, many research groups have assessed RIBE in *in vitro* and *in vivo* models. Han et al. observed significantly increased 53BP1 foci formation in proliferating bystander Chinese Hamster Ovary (CHO) cells, which were co-cultured with cells