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Participation of gap junction communication in potentially lethal damage repair and DNA damage in human fibroblasts exposed to low- or high-LET radiation

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ABSTRACT

Existing research has not fully explained how different types of ionizing radiation (IR) modulate the responses of cell populations or tissues. In our previous work, we showed that gap junction intercellular communication (GJIC) mediates the propagation of stressful effects among irradiated cells exposed to high linear energy transfer (LET) radiations, in which almost every cells is traversed by an IR track. In the present study, we conducted an in-depth study of the role of GJIC in modulating the repair of potentially lethal damage (PLDR) and micronuclei formation in cells exposed to low- or high-LET IR. Confluent human fibroblasts were exposed in the presence or absence of a gap junction inhibitor to 200 kV X rays (LET ~ 1.7 keV/μm), carbon ions (LET ~ 76 keV/μm), silicon ions (LET ~ 113 keV/μm) or iron ions (LET ~ 400 keV/μm) that resulted in isosurvival levels. The fibroblasts were incubated for various times at 37 °C. As expected, high-LET IR were more effective than were low-LET X rays at killing cells and damaging DNA shortly after irradiation. However, when cells were held in a confluent state for several hours, PLDR associated with a reduction in DNA damage, occurred only in cells exposed to X rays. Interestingly, inhibition of GJIC eliminated the enhancement of toxic effects, which resulted in an increase of cell survival and reduction in the level of micronucleus formation in cells exposed to high, but not in those exposed to low-LET IR. The experiment shows that gap-junction communication plays an important role in the propagation of stressful effects among irradiated cells exposed to high-LET IR while GJIC has only a minimal effect on PLDR and DNA damage following low-LET irradiation. Together, our results show that PLDR and induction of DNA damage clearly depend on gap-junction communication and radiation quality.

1. Introduction

It is now generally accepted that ionizing radiation (IR)-induced lethal damage can be attenuated by appropriate post-irradiation treatment conditions [1,2]. Holding X-irradiated cells in the confluent state for several hours after irradiation significantly enhanced their survival [3]. This protective effect is due to the repair of potentially lethal damage (PLD) [3,4]. Most PLD repair (PLDR) studies have been performed in mammalian cells exposed to low linear energy transfer (LET) radiation such as X and γ rays [5,6]; fewer studies have observed this phenomenon in cells exposed to high-LET IR such as α particles, high-charged, high-energy (HZE) particles or heavy ions [6–8]. While much is known about factors influencing PLDR, comparatively little is known about the underlying mechanisms involved, especially in the case of cells exposed to high-LET IR. This is often explained by the hypothesis that the repair mechanisms are less effective with lesions generated by high-LET IR [4]. In general, high-LET IR induces clustered DNA damage is less easily repaired and subsequently leads to a more efficient cell killing than low-LET IR [9–11] However, the exact mechanisms occurring in cells irradiated by high-LET IR remain undefined and are likely to depend on cell-to-cell communication [12–14].

During the past decade, several mechanisms have been discovered in the propagation of IR-induced damaging effects in cells

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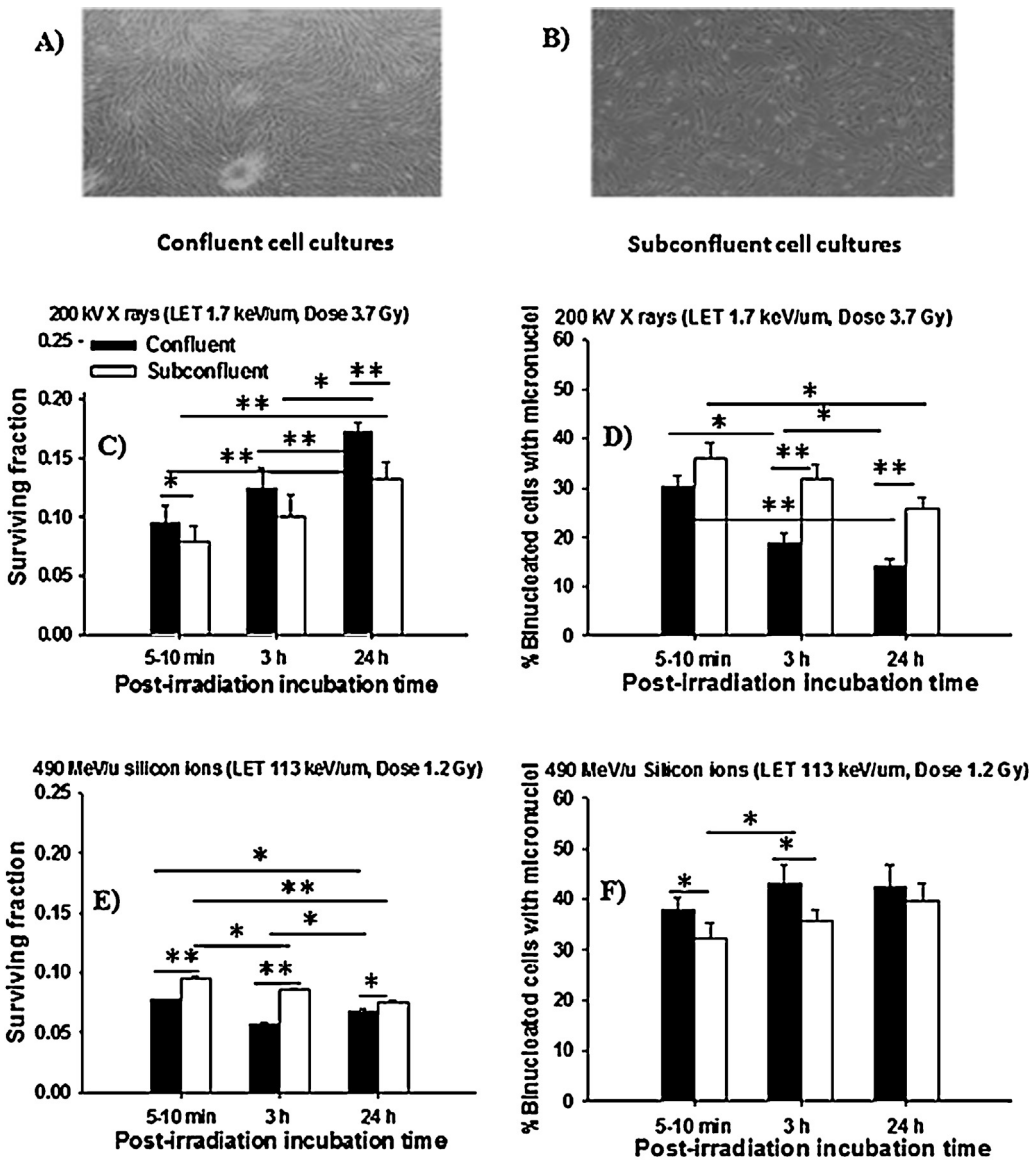

Fig. 1. Gap junction intercellular communication in the propagation of stressful effects among NB1RGB human cells exposed to low-LET X rays and high-LET silicon ions followed by 5–10 min, 3 h or 24 h incubation at 37 °C and held in a confluent or subconfluent state. (Panel (A), confluent cell cultures; Panel (B), subconfluent cell cultures; Panel (C), clonogenic survival of confluent and subconfluent cells after exposure to X rays; Panel (D), fraction of micronucleated cells in irradiated cells held in a confluent or subconfluent state after irradiation with X rays; Panel (E), clonogenic survival of confluent and subconfluent cells exposed to silicon ions; Panel (F), fraction of micronucelated cells in irradiated cells held in a confluent or subconfluent state exposed to silicon ions) ($^{*}p < 0.05$; $^{**}p < 0.01$).

exposed to low- or high-LET IR. They include intercellular communication via the gap-junction and/or secreted cytotoxic factors from culture medium, perturbations of oxidative metabolism, and other mechanisms [15–17]. Gap junction intercellular communications (GJIC) linking adjacent cells were shown, by direct approaches, to mediate the propagation of toxic effects among irradiated cells [13,14] and between irradiated and non-irradiated bystander cells [15–18]. Whether they contribute to the propagation of stressful or protective effects among irradiated cells with low- or high-LET IR is not well understood.

Gap-junctions are dynamic structures that are critical for diverse physiological functions [19]. The intercellular channels that comprise gap-junctions are formed by connexin protein, and each of the ~20 isoforms of connexins forms channels with distinct permeability properties [19]. By allowing direct intercellular communication between ions and low-molecular weight molecules,

Table 1
Dosimetry parameters for NB1RGB-irradiated cells exposure to high-LET radiations.

Radiation	Initial energy (MeV/u)	Dose (Gy)	Fluence (particle/cm^2)	LET[a] (keV/μm)	Unhit fraction	Hit fraction	Average hits (per cell nucleus)	Inactivation cross-section (μm^2)
Carbon ions	290	1.4	1.14×10^7	76.0	0	1	20	8.69
Silicon ions	490	1.2	6.63×10^6	113.0	0	1	10	15.08
Iron ions	500	1.3	2.02×10^6	400.0	0.03	0.97	3	49.29

[a] The LETs at the sample position.