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Radiation-induced bystander effects enhanced by elevated sodium chloride through sensitizing cells to bystander factors

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ABSTRACT

Radiation-induced bystander effects (RIBE) have been demonstrated to occur widely in various cell lines. However, very little data is available on the genotoxic effects of RIBE combined with other factor(s). We reported previously that with a low dose of α-particle irradiation, the fraction of γ-H2AX foci-positive cells in non-irradiated bystander cells was significantly increased under elevated NaCl culture conditions. In this study, we further investigated the functional role of NaCl in the enhancement of RIBE using a specially designed co-culture system and micronucleus (MN) test. It was shown that the MN frequency was not increased significantly by elevated NaCl (9.0 g/L) alone or by medium exposure. However, with 1.0 cGy α-particle irradiation, the induced MN frequency increased significantly in both irradiated and non-irradiated bystander regions. Additional studies showed that elevated NaCl made the non-irradiated bystander cells more vulnerable to bystander factors. Furthermore, it was found that the induced MN frequency in cells both in irradiated and non-irradiated bystander regions was weakened when the hypertonic medium was changed to normotonic medium for 2 h before irradiation. Such observations were quite similar to the co-effect of NaCl and hydrogen peroxide (H_2O_2), indicating that elevated NaCl might sensitize non-irradiated cells to bystander factors-induced oxidative stress.

1. Introduction

Ever since radiation-induced bystander effects (RIBE) were described as early as 1947 [1], there has been increasing evidence for the existence of these phenomena. Nagasawa and Little [2] first demonstrated a bystander effect induced in cell cultures exposed to low-dose α-particle irradiation. So far, RIBE have been well demonstrated with a variety of biological endpoints in both human and rodent cell lines [3–7]. Subsequent studies have confirmed that there are two major types of bystander effects. The first depends on cell/cell communication and the second results from substances released from the exposed cell to the medium [3]. The important roles of gap junction intercellular communication [8] and soluble factors like reactive oxygen species (ROS) [8,9], nitric oxide (NO) [10,11] and cytokines released from irradiated cells in RIBE have been reported recently [5]. This extensive research also implies that in addition to nucleus irradiation, cytoplasmic irradiation can also induce RIBE [12,13]. However, less evidence is available on the co-effects of RIBE and other factor(s).

The genotoxic effects of NaCl have been supported strongly by many studies. Elevating NaCl can induce osmotic stress and the changes in extracellular osmolality impair cell metabolism and function in a variety of ways [14,15]. It has been reported that an acute elevation of NaCl concentration causes DNA double-strand breaks (DSBs) [16] and inhibits DNA repair [17–19]. Other effects include changes in cell shape, disturbances in biochemical reactions, cell cycle arrests, induction of apoptosis, reduction in the number of cells, and the rate at which cells grow [15,19–21]. These effects increase the probability that NaCl will alter DNA damaging effects of other genotoxic agents. It has been shown that post-irradiation hypertonic treatment increases the formation of chromosome aberrations and cell death, as well as reduces DNA synthesis and double strand break repair [22–25]. Our recent experiment indicated that the fraction of γ-H2AX foci-positive cells was significantly increased in cells both in irradiated and non-irradiated regions under the elevated NaCl culture condition [26]. However, the underlying mechanism is not clear and how elevated NaCl affects RIBE remains to be addressed.

In the present study, based on the MN test and a specially designed detachable co-culture system, we further investigated RIBE in normal human fibroblasts by acute exposure to elevated

Abbreviations: RIBE, radiation-induced bystander effects; MN, micronuclei; ROS, reactive oxygen species; NO, nitric oxide; DSB, DNA double-strand break; γ-H2AX, phosphorylated form of H2AX protein.

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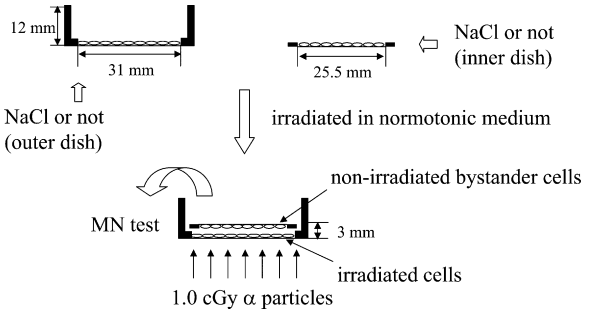

Fig. 1. A special detachable co-culture system was utilized in present study. It consists of outer and inner dishes. Both dishes are made of stainless steel and each of them has a base made of mylar film that is 3.5 μm thick. The diameters of outer and inner dish are 31.0 and 25.5 mm, respectively. There is a stage at the outer dish bottom which is 2.0 mm high. The height of the inner dish is 1.0 mm. The co-culture was done by putting the inner dish into the outer dish upside down so that the cells in the inner dish could co-culture with the cells in the outer dish "face-to-face". Before irradiation, the cells in the outer and inner dishes were treated with elevated NaCl concentration simultaneously or separately depending on different experiments. After irradiation, the irradiated and bystander cells were co-cultured for 3 h in the 37 °C incubator, and followed by MN test.

NaCl for 12 h before irradiation. We found that with 1.0 cGy α-particle irradiation, a significant increase of MN was detected in cells both in irradiated and non-irradiated bystander regions under elevated NaCl culture condition. Subsequent results showed that elevated NaCl treatment made bystander cells more vulnerable to radiation induced bystander factors, and such an effect might result from the increased sensitivity of non-irradiated cells to oxidative stress.

2. Material and method

2.1. Cell culture and co-culture system

AG 1522 normal human diploid skin fibroblasts kindly provided by Dr. Barry Michael (Gray Cancer Institute, UK) were maintained in α-Eagle's minimum essential medium (Gibco, Grand Island, NY, USA) supplemented with 2.0 mM L-glutamine (Gibco, Grand Island, NY, USA) and 20% FBS (Hyclone, Logan, UT, USA) plus 100 μg/ml streptomycin and 100 U/ml penicillin (Gibco, Grand Island, NY, USA) at 37 °C in a humidified 5% CO_2 incubator (SanYo, Japan).

The co-culture system was designed as described with some modification [27–32]. Briefly, it consists of outer and inner dishes (Fig. 1). Both dishes are made of stainless steel and each of them has a base made of mylar film that is 3.5 μm thick. Approximately, 1×10^4 and 1×10^5 exponentially growing AG 1522 cells in passage 11–14 were seeded in each outer and inner dish, which were cultured in 60 and 35 mm culture dishes separately. The culture medium was replaced every 2 days and 100% confluent cultures were used for irradiation. The co-culture was done by putting the inner dish into the outer dish upside down. The cells in the inner dish (inner-dish-cells) could co-culture with the cells in the outer dish (outer-dish-cells) "face-to-face". The distance between them is about 3 mm. Since 3.5 MeV α-particles can traverse only a very limited distance (~24 μm) in medium, the inner-dish-cells can not be irradiated while the outer-dish-cells are hit by α-particles.

2.2. α-Particles irradiation and treatment of outer and inner cells with elevated NaCl or H_2O_2

The average energy and LET of α-particles derived from ^{241}Am irradiation source, measured at the cell surface, was 3.5 MeV and 128 keV/μm and the particles were delivered at a dose rate of 1.0 cGy s^{-1} [26,33]. The normal concentration of NaCl in AG 1522 culture medium was 6.8 g/L and hypertonic medium (9.0 g/L) was prepared by adding high concentrated NaCl solution prepared with double-distilled water to normal culture medium 12 h before irradiation. The control sample was added equal volume of double-distilled water prepared NaCl solution (6.8 g/L).

Before irradiation, the cells in the outer and inner dishes were treated with elevated NaCl culture medium simultaneously or separately depending on different experiments. After NaCl treatment, the hypertonic medium was aspirated and the cells were washed with PBS (phosphate buffered saline). The inner dishes were then put into the outer dishes quickly. At the same time, normotonic medium was added. The cells in the outer dish were irradiated immediately or 2 h later. After irradiation, the cells in the outer and inner dishes were co-cultured for 3 h in the 37 °C incubator, which was followed by MN test. In some cases, the outer dishes without cells were irradiated to observe any effect on inner-dish-cells by medium exposure.

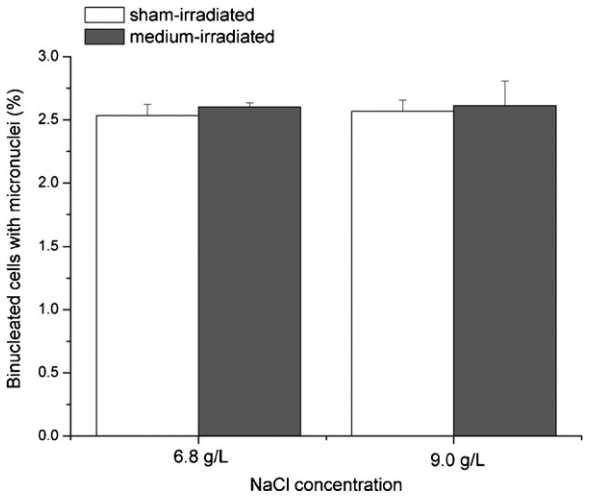

Fig. 2. Induction of MN frequency in cells by elevated NaCl concentration (9.0 g/L) alone or by medium irradiation with 1.0 cGy dose of α-particles. Results showed that the frequency of MN did not increase significantly when the cells were treated with 9.0 g/L NaCl alone even for 12 h and radiation of outer dish medium had no effects on the MN induction in the inner-dish-cells whether treated with elevated NaCl concentration or not.

To investigate the role of oxidative stress in the NaCl-induced enhancement of RIBE, H_2O_2 was selected to simulate the RIBE-induced oxidative stress. 1×10^5 cells were seeded in 35 mm culture dishes and cultured for 2 days. Some of them were then treated with elevated NaCl concentration (9.0 g/L) for 12 h. At the end of NaCl treatment, the cells were exposed to 10 μM H_2O_2 for 3 h immediately or 2 h later.

2.3. Micronucleus test

Three hours after irradiation or H_2O_2 treatment, cells were trypsinized and prepared for micronucleus test using the cytokinesis-block technique as described [34]. The number of MN in at least 1000 binucleate (BN) cells was scored and the frequency of MN per 1000 BN cells was calculated. The induced binucleated cells with micronuclei were adopted in Figs. 3–5 to access the additive effects of elevated NaCl concentration with radiation or H_2O_2 by eliminating the influence of elevated NaCl concentration alone on the induction of background of MN induction.

2.4. Statistics

All data were pooled from at least three independent experiments, and the results are presented as means ± S.D. Significance was assessed using Student's *t*-test. A *p*-value of ≤0.05 between groups is considered significant.

3. Results

3.1. Exposure to 9.0 g/L NaCl or irradiated medium alone does not increase the MN frequency

To examine any effect on cells by medium exposure, outer dish mylar film was irradiated without attached cells. The MN frequency of non-irradiated cells grown in the inner dishes was determined. It was found that irradiated medium had no effects on the MN induction in the inner-dish-cells even when treated with elevated NaCl. As shown in Fig. 2, the frequencies of MN in normal and NaCl-treated cells were 2.533 ± 0.088% and 2.567 ± 0.088%, respectively. With incubation in irradiated-medium, the MN frequencies became 2.600 ± 0.033% and 2.611 ± 0.195%, showing no significant changes over the corresponding controls. Moreover, the elevated NaCl concentration of 9.0 g/L alone was demonstrated in the present study not to increase the background MN frequency.