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# Role of ATM in bystander signaling between human monocytes and lung adenocarcinoma cells

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ABSTRACT

The response of a cell or tissue to ionizing radiation is mediated by direct damage to cellular components and indirect damage mediated by radiolysis of water. Radiation affects both irradiated cells and the surrounding cells and tissues. The radiation-induced bystander effect is defined by the presence of biological effects in cells that were not themselves in the field of irradiation. To establish the contribution of the bystander effect in the survival of the neighboring cells, lung carcinoma A549 cells were exposed to gamma-irradiation, 2 Gy. The medium from the irradiated cells was transferred to non-irradiated A549 cells. Irradiated A549 cells as well as non-irradiated A549 cells cultured in the presence of medium from irradiated cells showed decrease in survival and increase in γ-H2AX and p-ATM foci, indicating a bystander effect. Bystander signaling was also observed between different cell types. Phorbol-12-myristate-13-acetate (PMA)-stimulated and gamma-irradiated U937 (human monocyte) cells induced a bystander response in non-irradiated A549 (lung carcinoma) cells as shown by decreased survival and increased γ-H2AX and p-ATM foci. Non-stimulated and/or irradiated U937 cells did not induce such effects in non-irradiated A549 cells.

Since ATM protein was activated in irradiated cells as well as bystander cells, it was of interest to understand its role in bystander effect. Suppression of ATM with siRNA in A549 cells completely inhibited bystander effect in bystander A549 cells. On the other hand suppression of ATM with siRNA in PMA stimulated U937 cells caused only a partial inhibition of bystander effect in bystander A549 cells. These results indicate that apart from ATM, some additional factor may be involved in bystander effect between different cell types.



## 1. Introduction

The target for the biological effects of ionizing radiation includes not only the irradiated cell, but also surrounding cells (bystander cells) and tissues. The "bystander effect" occurs when a directly irradiated cell generates and transmits a signal to a neighboring non-irradiated cell. Several factors, such as reactive oxygen species (ROS) [1], nitric oxide species [2,3], and cytokines [4], have been implicated in the bystander effect. These factors may be transmitted either through gap junctions [5,6] or through the medium [7,8] to a neighboring non-irradiated cell. Multiple biological endpoints have been used to study the bystander effect: micronucleus formation [9], clonogenic survival [10], apoptosis [11], and sister chromatid exchanges (SCE) [12]. These biological markers are themselves considered to be detrimental; it has been assumed that the bystander effect is harmful to neighboring cells.

In contrast to our understanding of DNA damage induced by direct irradiation, the mechanism of DNA damage in the bystander cell is still poorly understood. The study of signal transduction networks between irradiated cells and neighboring non-irradiated cells, resulting in the induction of DNA and chromosomal damage in non-irradiated cells, is relatively new. Radiotherapy, which is a highly effective modality of cancer treatment, is based on the ideas that direct radiation exposure leads to critical DNA damage which ultimately results in tumor cell death. Various DNA damage repair and cell cycle control proteins have attracted attention as molecular targets for the treatment of malignant tumors in combination with chemotherapy and radiotherapy. The potential role of bystander signaling in cancer therapy has been discussed [13,14].

However, it has been increasingly recognized that non-targeted irradiation or abscopal effects are responsible for retarding the growth of a similar tumor located at a distant site in mice and in human patients with melanoma [15,16]. The contributions of

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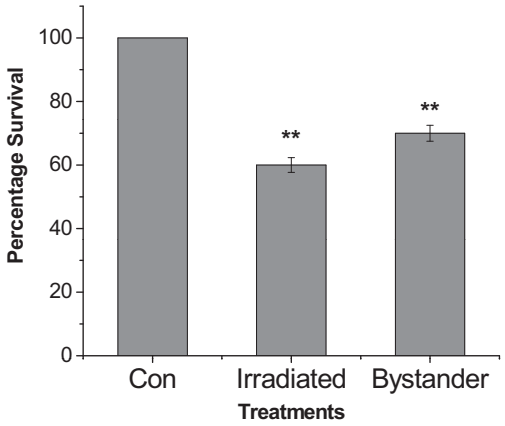


**Fig. 1.** Clonogenic cell survival of A549 cells in the presence of different conditioned media. Key: Lane 1, control non-irradiated A549 cells; Lane 2, γ-irradiated A549 cells; Lane 3, non-irradiated A549 cells receiving medium from γ-irradiated A549 cells. Data represent means ± SE of three independent experiments; significantly different from non-irradiated controls: $^{**}p < 0.01$.

abscopal effects in transferring signals between dissimilar cells have not been extensively studied. Activation of the immune system has been proposed as the major component of abscopal effects after radiotherapy [16].

We have studied the mechanism of the bystander response in similar (A549 vs A549) and dissimilar (U937 vs A549) cell types. To model abscopal effects in vitro, we have used two dissimilar cell lines viz. A549 cells, a human lung adenocarcinoma cell line and U937, a human monocyte cell line. Since there is extensive intercellular communication between dissimilar cells during the immune response, the lung carcinoma and lymphocytes were deemed suitable candidates to study bystander effects.

Several studies have demonstrated the importance of DNA repair proteins in directly irradiated cells. In this study, we sought to examine what, if any, role these proteins might play in the bystander effect. Here, we focused on the repair protein ATM (Ataxia Telangectasia Mutated), which is the most proximal signal transducer initiating cell cycle changes after DNA damage induced by ionizing radiation [17].

## 2. Materials and methods

### 2.1. Cell culture and irradiation schedule

The human lung adenocarcinoma cell line A549 and human monocyte U937 cells were grown in RPMI 1640 medium (Sigma, St. Louis, MO), supplemented with 10% fetal calf serum (Sigma, St. Louis, MO). Cells were maintained at 37 °C in humidified atmosphere with 5% $CO_2$.

For irradiation, A549 and U937 cells were resuspended in RPMI medium at a density of $1 \times 10^6$ cells/ml and exposed to 2 Gy γ-irradiation using Gamma Cell 220 irradiator (Atomic Energy of Canada, Ltd.), at a dose rate of 3 Gy/min. One hour after irradiation, medium from irradiated cells was transferred to same numbers of non-irradiated cells (at a density of $1 \times 10^6$ cells/ml). A group of U937 cells were stimulated with 32 nM phorbol-12-myristate-13-acetate (PMA, Sigma, St. Louis, MO) for 24 h and then the medium was replaced with fresh medium without PMA and then irradiated.

The experimental design consisted of the following sets: (a) control non-irradiated cells, (b) γ-irradiated cells, (c) A549 cells receiving medium from non-irradiated A549 cells or U937 cells or PMA-stimulated U937 cells, (d) A549 cells receiving medium from γ-irradiated A549 cells or irradiated U937 cells or PMA-stimulated and irradiated U937 cells, and (e) A549 cells receiving γ-irradiated medium. Cells (A549 and PMA-stimulated U937) were transfected with ATM siRNA and irradiated media from these cells were transferred to non-irradiated A549 cells.

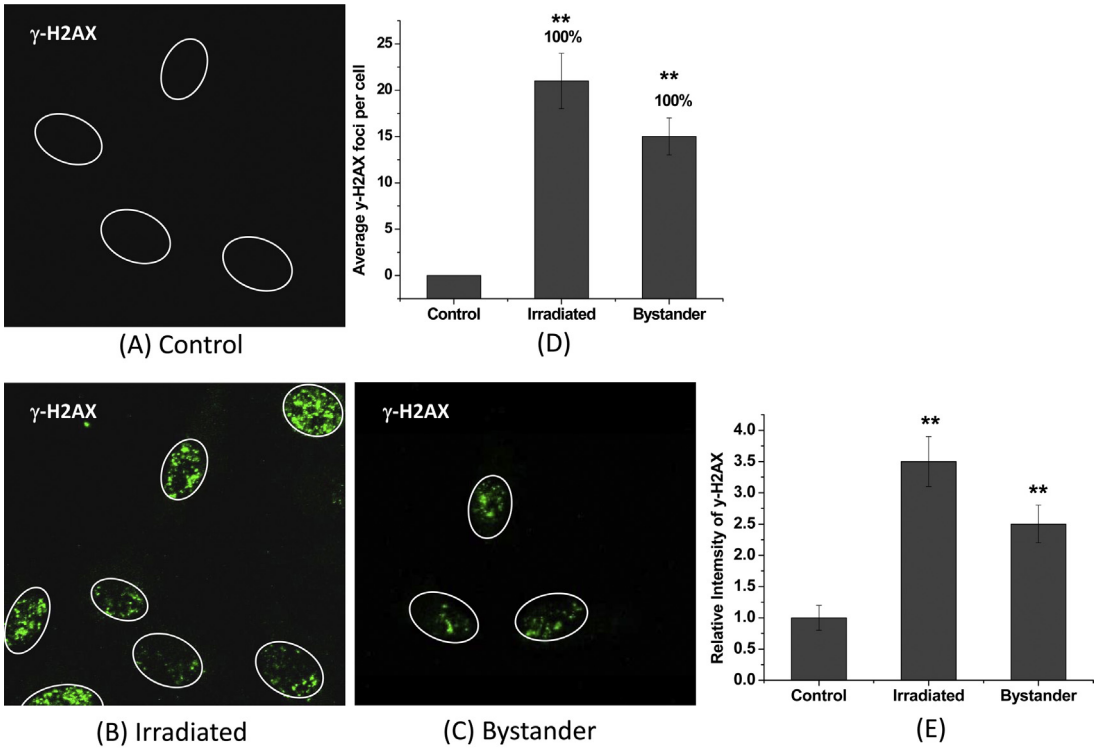


**Fig. 2.** Radiation induced γ-H2AX foci of A549 cells cultured for 15 min in the presence of different conditioned media. Cells were fixed 15 min after treatment in 4% paraformaldehyde, permeabilized in 0.25% Triton X-100 and labeled with specific antibodies as described in Section 2. Each phospho-site antibody was indirectly labeled with Molecular Probe 488 secondary antibody (green) and cells were mounted with ProLong Gold antifade with DAPI (blue). The encircled area roughly represents cell nuclei as seen by DAPI staining. All images were captured using Carl Zeiss confocal microscope with the same exposure time. (A) control non-irradiated A549 cells; (B) γ-irradiated A549 cells; (C) non-irradiated A549 cells receiving medium from γ-irradiated A549 cells; (D) graph represents average numbers of foci per cell in control, irradiated and bystander cells, percentage of cells showing the foci is marked above the bars. (E) Graph represents relative intensity of γ-H2AX as determined by ImageJ software in control, irradiated and bystander cells. At least 100 cells per experiment were analyzed from three independent experiments. Data represent means ± SE of three independent experiments; significantly different from non-irradiated controls: $^{**}p < 0.01$.