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Bystander Effectors of Chondrosarcoma Cells Irradiated at Different Let Impair Proliferation of Chondrocytes

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Abstract: Radiation-induced bystander effects (RIBE) describe non-targeted cellular responses in non-irradiated cells influenced by signals from irradiated neighbors. Although documented in various systems, their mechanisms in chondrosarcoma–chondrocyte interactions, especially under high linear energy transfer (LET) radiation, remain poorly understood. This study evaluated and compared the direct and bystander responses of human chondrocytes (T/C-28a2) exposed to factors released by irradiated human chondrosarcoma cells (SW1353) using low-LET X-rays and high-LET carbon ions (C-ions). SW1353 cells were irradiated with 0.05–8 Gy of X-rays or C-ions, and conditioned medium was transferred to non-irradiated T/C-28a2 cells. Endpoints included clonogenic survival, proliferation (impedancemetry), DNA damage (micronucleus assay), and cytokine profiling. The effects of conditioned medium dilution and heat treatment were also examined. Low doses (0.1 Gy X-rays, 0.05 Gy C-ions) elicited maximal bystander effects, reducing chondrocyte survival to 36% and 62%, respectively. DNA damage was significantly increased at these doses. Dilution ≥50% or heating to ≥70 °C abolished bystander effects. TNF-α and IL-6 were identified as key cytotoxic and genotoxic effects in chondrocytes at low radiation doses. These findings highlight the clinical need to consider RIBE in high-precision therapies.

Keywords: Radiation-induced bystander effects (RIBE), linear energy transfer (LET), impedancemetry, micronucleus.

1. INTRODUCTION

Chondrosarcoma is a common bone tumor with a cartilaginous extracellular matrix and resistance to chemotherapy and radiotherapy (Boissonnat G, 2017, Durante M, Debus J (2018), Smith (2018)). Surgical resection is the primary treatment, but inoperable cases require adjuvant radiotherapy (Lepleux, **2019**). Hadrontherapy using carbon ions is increasingly considered for radioresistant tumors due to its advantages over X-rays (Banani (2017), Guzikowski 2019), Mahboubi (2017) . Despite the physical precision, biological cross-talk between irradiated and surrounding non-irradiated cells can compromise treatment specificity via radiation-induced bystander effects (RIBE) (Gallego-García (2019), Martínez-Martínez (2017), Chevalier (2019)). RIBE refers to radiation-like responses in non-irradiated cells resulting from intercellular communication through gap junctions or soluble mediators such as cytokines, reactive oxygen/nitrogen species, and extracellular vesicles (Shakeri Manesh (2017), Lamuraglia M (2020)). RIBE, a cytokine, is influenced by radiation quality, dose, cell type, and genetic status, often triggered by NF-κB and MAPK signaling cascades.

LITERATURE REVIEW:

Radiation-induced bystander effects (RIBE) are damage caused by intercellular signaling from irradiated cells (Martínez-Martínez (2017), Chevalier (2019)). The nature of RIBE is influenced by radiation quality, with low-LET X-rays causing transient responses and high-LET radiations triggering complex signaling. Cytokines activate NF-κB and MAPK pathways, leading to genomic instability and altered proliferation. Studies in musculoskeletal systems are limited, but mesenchymal cells are particularly susceptible.

MATERIALS AND METHODS

The study involved cultured human chondrosarcoma SW1353 cells and immortalized human juvenile chondrocytes T/C-28a2 in a humidified atmosphere. X-ray irradiations were performed using a 225 kV source, and C-ion irradiations were conducted at GANIL and HIMAC. The cells were incubated with fresh medium for 24 hours to accumulate secreted factors, and then transferred to confluent T/C-28a2 cultures for 24 hours.

RESULTS AND DISCUSSION:

Clonogenic assays demonstrated a dose-dependent decrease in T/C-28a2 survival following direct irradiation by both modalities (Fig.1). C-ions induced greater cytotoxicity compared to X-rays at equivalent doses. The D_{10} values were 3.4 Gy for X-rays and 1.36 Gy for C-ions, corresponding to an RBE of 2.49; the D_{37} RBE was 3.58. Micronucleus (MN) formation increased proportionally with dose for both modalities, with C-ions producing higher MN yields (RBE ≈ 2 at 1–2 Gy). Conditioned medium from SW1353 cells irradiated at low doses caused marked survival reduction in T/C-28a2 cells. At 0.1 Gy X-rays, survival dropped to 36 ± 4%, while at 0.05 Gy C-ions survival was 62 ± 6% ($p < 0.05$ vs control).

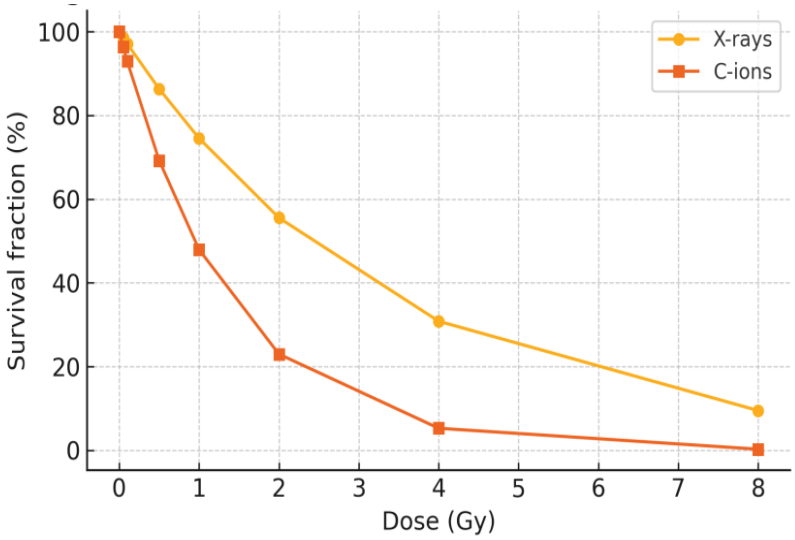

Figure 1. Survival of T/C-28a2 after direct irradiation

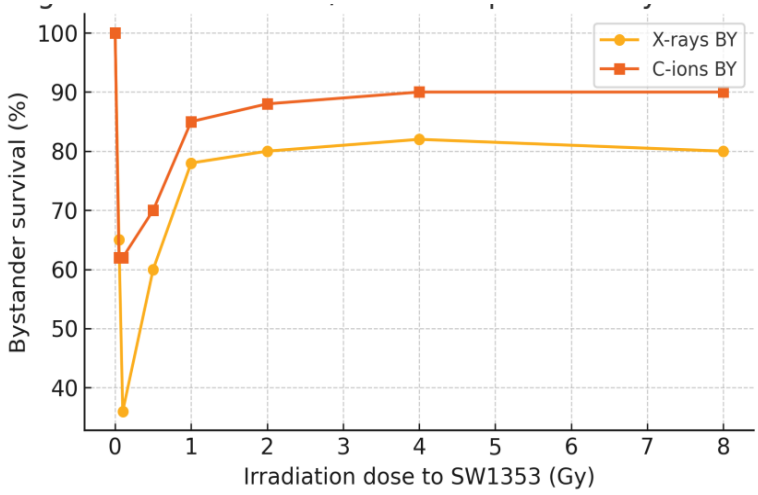

Figure 2. Survival of T/C-28a2 exposed to bystander CM

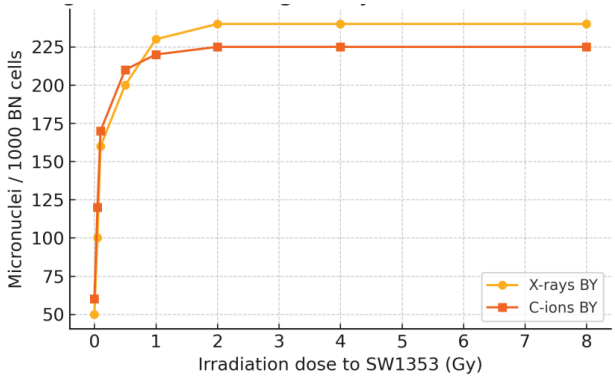

Figure 3. DNA damage in bystander T/C-28a2 cells

At higher doses (≥1 Gy), survival reductions plateaued at ~80% (X-rays) and ~90% (C-ions). Impedancemetry showed significant proliferation delays in T/C-28a2 cells exposed to bystander CM from 0.1 Gy X-rays and from 0.5–8 Gy C-ions (Fig.2). Declines in cell index were evident within 24 h and persisted up to 48 h. MN frequencies in bystander chondrocytes increased from 0.1 Gy (X-rays) and 0.05 Gy (C-ions) upwards, plateauing at ~240 MN/1000 BN cells for X-rays and ~225 MN/1000 BN cells for C-ions. Dilution to 50% and 25% CM attenuated the bystander effect; 10% CM abolished it. Heat treatment at 70 °C and 95 °C completely eliminated the effect, indicating the mediators are thermolabile proteins. TNF-α levels in CM increased 1.5-fold after 0.1 Gy X-rays and 3.2-fold after 0.1 Gy C-ions. IL-6 was significantly elevated only at higher doses (2 Gy X-rays: 2.4-fold). IL-1β and IL-8 showed no significant changes. Exogenous TNF-α treatment reproduced survival reduction in chondrocytes, implicating TNF-α as a key mediator. This study confirms that irradiated chondrosarcoma cells release factors capable of impairing survival and proliferation, and inducing DNA damage in non-irradiated chondrocytes. The most pronounced effects occurred at very low doses (<0.1 Gy), consistent with other RIBE reports describing a non-linear dose–response. Direct effects were stronger for C-ions due to their high-LET nature, which produces dense ionization and complex DNA damage (Banani (2017), Guzikowski 2019), Mahboubi (2017). However, bystander effects were more pronounced for X-rays at equivalent low doses. This may reflect differences in signaling molecule release kinetics or oxidative stress induction between radiation qualitiesThe thermolabile nature of bystander activity and its loss upon dilution point toward proteinaceous mediators, primarily cytokines. Elevated TNF-α at low doses and IL-6 at higher doses aligns with NF-κB-driven inflammatory responses documented in other RIBE systemsRecent findings show TNF-α and TGF-β1 regulate both early and persistent RIBE in mesenchymal stem cells, while proteomic profiling implicates stress granules and vesicular transport in mediator release. Our findings suggest that in carbon ion therapy for chondrosarcoma, even adjacent healthy cartilage may experience biological effects despite physical dose sparing. This has implications for planning margins, dose fractionation, and possible use of anti-inflammatory agents or cytokine inhibitors to mitigate RIBE. The immortalized, p53-deficient status of T/C-28a2 cells may affect sensitivity to bystander factors. Validation in primary chondrocytes and in vivo models is warranted. Additionally, while TNF-α and IL-6 were identified, the role of other mediators such as exosomes, ROS, or microRNAs warrants investigation.

5. CONCLUSION

Irradiated chondrosarcoma cells release TNF-α and IL-6, among other thermolabile factors, that significantly impair survival and proliferation, and induce genomic damage in non-irradiated chondrocytes at very low doses. These results highlight the need to integrate RIBE considerations into the radiotherapy planning for cartilage-associated tumors, even with high-precision modalities such as carbon ion therapy.

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