FROM HEAD TO TOE: THE CELLULAR RESPONSE TO HEAVY ION EXPOSURE

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INTRODUCTION

As a prerequisite for developing appropriate countermeasures to mitigate acute effects and late radiation risks for the astronaut and thereby enabling long-term human space flight, the cellular radiation response to densely ionizing radiation needs to be better understood. The biological effectiveness of accelerated heavy ions with high linear energy transfer (LET) for effecting DNA damage response pathways as a gateway to cell death or survival is of major concern for space missions. As body tissues differ in their radiation sensitivity, the radiation response of different cell types such as kidney cells, osteoblasts, fibroblasts, lens epithelial cells and of eyes lenses in culture was analyzed.

METHODS

Cells were exposed to heavy ions in a broad range of LET (0.3 - 9674 keV/µm) at the heavy ion accelerators GSI, Darmstadt, Germany, GANIL, Caen, France or HIMAC, Japan. X-rays were used as reference radiation. Survival was determined by the colony forming ability test. The relative biological effectiveness (RBE) of reduction of cellular survival was calculated for each radiation quality. Cell cycle progression was quantified by flow cytometry of propidium-iodide stained cells. Mineralization of extracellular matrix was determined by Alizarin Red Staining (ARS). DNA double strand breaks and their repair were visualized by γH2AX staining. Gene expression was analysed by reverse transcriptase quantitative real time PCR (RT-qPCR). Activation of Nuclear Factor κB (NF-κB) was determined by means of a NF-κB reporter cell line (HEK-pNF-κB-d2EGFP/Neo L2). The role of NF-κB in the cellular response to space-relevant radiation qualities was investigated by stable transfection with a short hairpin RNA plasmid targeting p65 for NF-κB knockdown.

RESULTS

A maximal RBE for cell killing was found for different cell types at an LET of ~ 150 keV/µm. Ions of this LET were also most effective in inducing cell cycle arrest when compared based on the survival level. NF-κB activation and NF-κB-dependent gene expression by heavy ions were highest in the LET range of 50-300 keV/µm. Stable knockdown of RelA resulted in higher sensitivity towards X-rays, but not towards heavy ions. NF-κB activation and NF-κB-dependent gene expression occurred as an early step in the cellular radiation response. The expression of several chemokines and cytokines (CXCL1, CXCL2, CXCL10, IL-8 and TNF) was up-regulated and the extent of upregulation depended on LET. Cell-specific processes such as the differentiation of osteoblasts, including the mineralization process and Runx2 expression, were also modulated by heavy ion exposure. Lens epithelial cells, especially from the equatorial region of the eye lens, had particularly slow DNA repair with high residual damage.

CONCLUSION

The maximum RBE of heavy ions was observed in the LET range of 50-300 keV/µm for various cell types and biological endpoints (survival, cell cycle arrest, NF-κB activation). A sustained cell cycle arrest contributed strongly to reproductive cell death in this LET range. A survival advantage by NF-κB activation was only observed after X-irradiation and not after heavy ion exposure. The upregulated chemokines and cytokines might be important for cell-cell communication among hit as well as unhit cells (bystander effect). Their role in the cellular and tissue response to ionizing radiation needs to be further examined as they might induce proinflammatory effects. Furthermore, DNA repair impairment in the equatorial region of the eye lens might be responsible for the high radiosensitivity of the eye lens.

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One Sentence Summary:

The strong activation of pro-inflammatory signaling in human cells after heavy ion exposure might be a suitable target for countermeasure development.