

## Nuclear Factor $\kappa$ B (NF- $\kappa$ B) dependent responses and signals elicited by heavy ions 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 (which constitute the biologically most important radiation type in space) with high linear energy transfer (LET) for effecting DNA damage response pathways as a gateway to cell death or survival is of major concern not only for space missions but also for new regimes of tumor radiotherapy.

**Methods:** The contribution of NF- $\kappa$ B to the cellular response to space-relevant radiation qualities was determined by a NF- $\kappa$ B reporter cell line (HEK-pNF- $\kappa$ B-d2EGFP/Neo L2), by applying ATM or proteasome inhibitors, and by HEK cells stably transfected with a short hairpin RNA plasmid targeting p65 for NF- $\kappa$ B knockdown. The NF- $\kappa$ B dependent reporter gene expression (d2EGFP) after ionizing radiation (X-rays and heavy ions in broad range of LET: 0.3 - 9674 keV/ $\mu$ m) exposure was evaluated by flow cytometry. Survival was determined by the colony forming ability test. The biological effectiveness (RBE) of NF- $\kappa$ B activation and reduction of cellular survival were calculated for each radiation quality. Furthermore, the effect of LET on NF- $\kappa$ B target gene expression was analysed by real time reverse transcriptase quantitative PCR (RT-qPCR).

**Results:** Studies with chemical inhibitors showed that the DNA damage sensor and serine kinase ATM and the proteasome were essential for NF- $\kappa$ B activation in response to X-rays and heavy ions. Stable knockdown of RelA resulted in higher sensitivity towards X-rays, but not towards heavy ions. No differences were observed between untransfected and RelA knockdown cells in cell cycle progression under physiological conditions. There is only an extended lag-phase for RelA knockdown cells. A dose and radiation quality dependent arrest in the G2 phase of the cell cycle occurred in untransfected as well as in RelA knockdown cells. NF- $\kappa$ B activation and NF- $\kappa$ B dependent gene expression occurred as an early step in the cellular radiation response. NF- $\kappa$ B activation and NF- $\kappa$ B-dependent gene expression by heavy ions were highest in the LET range of 50-300 keV/ $\mu$ m. 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.

**Conclusion:** A survival advantage by NF- $\kappa$ B activation can only be 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.

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**Keywords:** Space Radiation, heavy ions, signal transduction, cytokines, chemokines

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