Transcriptional Response of Human Cells to Microbeam Irradiation with 2.1 MeV Alpha Particles

C. E. Hellweg (1), S.C. Bogner (1), L. Spitta (1), A. Arenz (1), **C. Baumstark-Khan** (1), K. Greif (2) and U. Giesen (2)

(1) DLR, Institut für Luft- und Raumfahrtmedizin, Strahlenbiologie, 51147 Köln, Germany,
(2) PTB, Microbeam and Ion Dosimetry Working Group, 38116 Braunschweig, Germany
(susanne.bogner@dlr.de / Fax: +49 2203-61970)

Within the next decades, an increasing number of human beings in space will be simultaneously exposed to different stimuli, especially microgravity and radiation. To assess the risks for humans during long-duration space missions the complex interplay of these parameters at the cellular level must be understood. Cellular stress protection responses lead to increased transcription of several genes via modulation of transcription factors. Activation of the *Nuclear Factor* κB (NF-κB) pathway as a possible anti-apoptotic route represents such an important cellular stress response. A screening assay for detection of NF-κB-dependent gene activation using the destabilized variant of *Enhanced Green Fluorescent Protein* (d2EGFP) as reporter protein had been developed. It consists of *Human Embryonic Kidney (HEK/293) Cells* stably transfected with a receptor-reporter-construct carrying d2EGFP under the control of a NF-κB response element. Clones positive for *Tumor Necrosis Factor* α (TNF-α) inducible d2EGFP expression were selected as cellular reporters. Irradiation was performed either with X-rays (150 kV, 19 mA) at DLR, Cologne, or with 2.1 MeV α particles (LET $\sim$160 keV/μm) at PTB, Braunschweig. After irradiation the following biological endpoints were determined (i) cell survival via the colony forming ability test, (ii) time-dependent activation of NF-κB dependent d2EGFP gene expression using flow cytometry, (iii) quantitative RT-PCR analysis of selected NF-κB target genes. Experiments using low-LET ionizing radiation (150 kV X-rays) at 0 $^{\circ}$C show only a slight but yet not significant increase of NF-κB dependent d2EGFP fluorescence after 5 Gy. For X-irradiation applied at 37 $^{\circ}$C, a significant dose-dependent increase in d2EGFP fluorescence can be verified. This may either reflect the dependence of NF-κB activation on free floating receptors or on DNA repair processes actively running already during irradiation. After exposure at 37 $^{\circ}$C of cell nuclei with one to ten α particles (2.1 MeV), d2EGFP fluorescence can already be seen 12 hours after exposure, with a maximum after 36 h. After exposure of HEK cell nuclei with 5 α particles, when maximal NF-κB activation is achieved, about 60 to 70 per cent of the irradiated cells survived. This exposure resulted in up-regulation of the expression of IκBα gene and down-regulation of CDKN1A. As activation of the NF-κB pathway is supposed to play a role in the negative regulation of apoptosis, survival of cells with DNA damage might be favoured especially after low doses of densely ionising radiation.