Suitability of Commonly Used Housekeeping Genes in Gene Expression Studies for Space Radiation Research

A. Arenz, C. E. Hellweg, S. Bogner, P. Lau, **C. Baumstark-Khan**

DLR, Institut für Luft- und Raumfahrtmedizin, Strahlenbiologie, 51147 Köln, Germany
(christa.baumstark-khan@dlr.de / Fax: +49 2203-61970)

Research on the effects of ionizing radiation exposure involves the use of real-time reverse transcription polymerase chain reaction (qRT-PCR) for measuring changes in gene expression. Several variables needs to be controlled for gene expression analysis – different amounts of starting material between the samples, variations in enzymatic efficiencies of the reverse transcription step and differences in RNA integrity. Normalization of the obtained data to an invariant endogenous control gene (reference gene) is the elementary step in relative quantification strategy. There is a strong correlation between the quality of the normalized data and the stability of the reference gene itself. This is especially relevant when the samples have been obtained after exposure to radiation qualities inducing different amounts and kinds of damage leading to a cell cycle delay or even to a cell cycle block. In order to determine suitable reference genes as internal controls in qRT-PCR assays after exposure to ionizing radiation, we studied the gene expression levels of commonly used reference genes in A549 lung cancer cells. Expression levels obtained for *human beta actin* (ACTB), *human beta-2-microglobulin* (B2M), *human glyceraldehyde-3-phosphate dehydrogenase* (GAPDH), *human porphobilinogen deaminase* (PBGD), *human 18S ribosomal RNA* (18S rRNA), *human glucose-6-phosphate dehydrogenase* (G6PDH), *human hypoxanthine phosphoribosyl transferase* (HPRT), *human ubiquitin C* (UBC), *human transferrin* (TFRC) and *human succinate dehydrogenase* (SDHA) were determined after exposure to 2 and 6 Gy X-radiation. Gene expression data for the radiation-inducible genes *Growth arrest DNA damage inducible gene 45* (GADD45α) and *p53-inducible ribonucleotide reductase* (RRM2B) were shown after normalization using either appropriate or inappropriate internal control genes. From these results we strongly recommend the use of a panel of reference genes instead of only one and to rely on the results obtained by using the three least variable genes for normalisation.