Murine Bone Cell Lines as Models for Spaceflight Induced Effects on Differentiation and Gene Expression

P. Lau , C.E. Hellweg , C. Baumstark-Khan , G. Reitz

German Aerospace Center, Institute of Aerospace Medicine, Cologne, Germany

(Patrick.Lau@dlr.de / Fax: (49)2203-61970 / Phone: (49)2203-6013173

Critical health factors for space crews especially on long-term missions are radiation exposure and the absence of gravity. DNA double strand breaks (DSB) are presumed to be the most deleterious DNA lesions after radiation as they disrupt both DNA strands in close proximity. Besides radiation risk, the absence of gravity influences the complex skeletal apparatus concerning muscle and especially bone remodelling which results from mechanical forces exerting on the body. Bone is a dynamic tissue, which is life-long remodelled by cells from the osteoblast and osteoclast lineage. Any imbalance of this system leads to pathological conditions such as osteoporosis or osteopetrosis. Osteoblastic cells play a crucial role in bone matrix synthesis and differentiate either into bone-lining cells or into osteocytes. Premature terminal differentiation has been reported to be induced by a number of DNA damaging or cell stress inducing agents including ionising and ultraviolet radiation as well as treatment with mitomycin C. In the present study, we compare the effects of sequential differentiation by adding osteoinductive substances (ß-glycerophosphate and ascorbic acid). Radiation-induced premature differentiation was investigated regarding the biosynthesis of specific osteogenic marker molecules and the differentiation dependent expression of marker genes. The bone cell model established in our laboratory consists of the osteocyte cell line MLO-Y4, the osteoblast cell line OCT-1 and the subclones 4 and 24 of the osteoblast cell line MC3T3-E1 expressing several differentiation potentials. Changes in bone cell morphology and functional protein level expression were investigated by means of histochemistry (alizarin red staining of calcium depositions), western blot analyses (osteocalcin synthesis) and immune fluorescent techniques (osteocalcin lokalisation). The induction and repair of DSB after irradiation were recognized by immunofluorescence detection of γH2AX antibodies and radiation dependent gene expression of e.g. GADD45 and RAD52. Differentiation will be investigated by gene expression studies using quantitative real-time reverse transcription PCR (qRT-PCR) for the bone specific markers genes OCN (osteocalcin) and OP (osteopontin).The presented bone cell model offers a new tool to investigate bone cell differentiation in combination with different radiation qualities. The interrelationship of differentiation status and radiation exposure may play an important role in bone formation and bone maintenance especially for astronauts concerning planed long-term mission.