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X-ray induced alterations in the differentiation and mineralization potential of murine preosteoblastic cells

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Abstract

To evaluate the effects of ionizing radiation (IR) on murine preosteoblastic cell differentiation, we directed OCT-1 cells to the osteoblastic lineage by treatment with a combination of β-glycerophosphate (β-GP), ascorbic acid (AA), and dexamethasone (Dex). *In vitro* mineralization was evaluated based on histochemical staining and quantification of the hydroxyapatite content of the extracellular bone matrix. Expression of mRNA encoding Runx2, transforming growth factor β1 (TGF-β1), osteocalcin (OCN), and $p21^{CDKN1A}$ was analyzed. Exposure to IR reduced the growth rate and diminished cell survival of OCT-1 cells under standard conditions. Notably, calcium content analysis revealed that deposition of mineralized matrix increased significantly under osteogenic conditions after X-ray exposure in a time-dependent manner. In this study, higher radiation doses exert significant overall effects on TGF-β1, OCN, and $p21^{CDKN1A}$ gene expression, suggesting that gene expression following X-ray treatment is affected in a dose-dependent manner. Additionally, we verified that Runx2 was suppressed within 24 h after irradiation at 2 and 4 Gy. Although further studies are required to verify the molecular mechanism, our observations strongly suggest that treatment with IR markedly alters the differentiation and mineralization process of preosteoblastic cells.

Keywords: Space; Ionizing radiation; Osteoblastic differentiation; Gene expression analysis

1. Introduction

During long-term space missions, the health of human space crew is affected by three major factors: ionizing space radiation, progressive loss of bone integrity due to bone demineralization, and adverse psychosocial reactions as a result of prolonged confinement and the "Earth-out-of-view" syndrome (Reitz et al., 2009). Physiological adaptations including bone loss, anemia, muscle atrophy, and immune alterations occur, at least in part, because of decreased activity of functional cells and reduced differentiation of progenitors into functional cells (Plett et al., 2004). During planned, long-term missions to the Moon and Mars, astronauts experience altered gravity and are exposed to galactic cosmic rays (Cucinotta and Durante, 2006). Space radiation produces distinct biological damage compared to radiation on Earth. This leads to uncertainties in risk projection for cancer and other health-related problems, and obscures evaluation of countermeasure effectiveness (Durante and Cucinotta, 2008).

Bone loss resulting from prolonged microgravity exposure has been the subject of investigation for several years (Rambout and Johnston, 1979). Previous studies have demonstrated that astronauts on 4- to 6-month missions aboard the International Space Station typically lose on

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the order of 1.2–1.5% of bone mass from their proximal femurs per month of spaceflight. Specifically, losses were associated with a decline in estimated bending and compression strength indices at these sites (Lang et al., 2004; Lang, 2006). Bone loss and the corresponding loss of strength may increase the risk of fractures and could pose a risk to mission safety. Skeletal integrity requires a dynamic balance between bone formation and bone resorption. Bone mass in mammals is governed by a bone-specific remodeling process comprised of the balanced actions of osteoblasts, cells found on bone surfaces that are responsible for bone formation, synthesis, deposition, and mineralization of the extracellular matrix (ECM) (Aubin, 1998; Mackie, 2003), and of osteoclasts, hematopoietic lineage-derived cells that are capable of bone resorption (Augat et al., 2005; Strewler, 2004).

Osteoblastic differentiation undergoes an ordered, time-dependent development sequence that leads to the formation of multilayered bone nodules (Seth et al., 2000). Three differentiation stages occur during osteoblastic differentiation and are defined as proliferation, matrix maturation, and mineralization (Mackie, 2003). Osteoblasts, which are derived from mesenchymal stem cells, synthesize organic matrix that forms a scaffold for mineralization. These cells are critical for establishing and maintaining bone structure. Further directed osteoblastic differentiation leads to either lining cells or osteocytes (Aubin et al., 1995; Aubin and Triffitt, 2002).

The effects of ionizing radiation (IR) on the skeletal system have not been fully defined; however, exposure to IR of different qualities has distinct, detrimental effects on bone microarchitecture in both cancer patients and in astronauts (Hamilton et al., 2006). The loss of bone mass after radiotherapy has been hypothesized to occur as a result of damage to osteoblasts and bone blood vessels (Sakurai et al., 2007; Willey et al., 2008). Inhibition of osteoblast differentiation and osteoblast progenitor differentiation after radiation exposure has been demonstrated both *in vitro* (Szymczyk et al., 2004) and *in vivo* (Sawajiri et al., 2003). These observations suggest that radiation affects immature osteoblasts more than mature ones. This supports the view that the cellular response to radiation depends largely on the differentiation status of the cell (Dare et al., 1997).

A better understanding of the response of bone cells, particularly during differentiation and mineralization, to IR is important to protect astronauts from radiation during space travel as well as to protect patients during radiotherapy (Durante and Cucinotta, 2008). Previous studies suggest that IR-driven differentiation may result from a radiation-induced cell cycle arrest that modulates osteoblastic specific gene expression (Lau et al., 2010). This, in turn, leads to a decrease in osteoblastogenesis and osteoblastic activity and function, ultimately resulting in a decrease in bone volume.

Very few studies have investigated the molecular mechanisms underlying cellular response to IR following osteogenic induction. In regards to this unique area of research, we evaluated cellular proliferation and mineralization as well as the mRNA expression of the osteoblast specific factors runt-related transcription factor 2 (Runx2), transforming growth factor β1 (TGF-β1), and osteocalcin (OCN) as well as $p21^{CDKN1A}$, in differentiating OCT-1 cells. TGF-β1 regulates a broad range of biological processes including cell proliferation, cell survival, cell differentiation, cell migration, and production of ECM (Moses and Serra, 1996; Massague et al., 2000; Verrecchia and Mauviel, 2002). Data from numerous *in vitro* experiments demonstrate the role of TGF-β1 in every stage of bone formation. Most data support the assumption that TGF-β1 increases bone formation *in vitro* predominately by recruiting and stimulating proliferation of osteoblast progenitors, thereby, expanding the pool of committed osteoblasts and promoting the early stages of differentiation (bone matrix production) (Janssens et al., 2005). Although nearly all cells synthesize and respond to TGF-β, bone and cartilage are especially rich in this particular growth factor (Lee et al., 2000). Additionally, various factors and signaling pathways downstream of TGF-β receptors are responsible for upregulation of the CDK inhibitor p21 gene and its promoter (Moustakas and Kardassis, 1998). Also of interest, Runx2 is the key transcription factor that drives mesenchymal precursor cell differentiation toward the osteoblastic lineage and controls bone formation. Runx2 regulates the expression of all known osteoblast markers (Ducy et al., 1997). The vitamin K-dependent protein OCN is expressed only post-proliferatively with the onset of nodule formation (Hauschka et al., 1989).

In this study, we examined alterations in the differentiation and mineralization processes of preosteoblastic cells after exposure to IR. We evaluated cell growth, proliferation, and osteoblastic differentiation based on the mineral content of the ECM. Our studies reveal that IR has no effect on the survival of cells induced towards the osteoblastic lineage through treatment with ascorbic acid (AA), β-Glycerophosphate (β-GP), and dexamethasone (Dex) compared to standard conditions. However, cells that were exposed to IR under osteogenic conditions displayed reduced mineral content of the ECM. We also demonstrate, by β-galactosidase (β-Gal) staining, that a senescent phenotype in OCT-1 cells occurs after exposure to IR in both standard and osteogenic conditions.

2. Materials and methods

2.1. Cell culture

We used low-passaged cells from the murine calvaria-derived osteoblast-like cell line, OCT-1 (kindly provided by D. Chen, San Antonio, TX) that is capable of differentiating along the osteoblast lineage (Lau et al., 2010) in this study. OCT-1 cells were originally isolated from transgenic mice CB6F1 (C57B1/6 × Balb/c) carrying the SV40 large T antigen under control of the bone specific OCN promoter