Impact of ionizing radiation on differentiation and proliferation of human osteoblasts

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Introduction

Bone metabolism is a highly regulated process in which the balance between bone-resorbing osteoclasts (OCs) and bone-forming osteoblasts (OBs) is influenced by hematopoietic and mesenchymal stem and progenitor cells (MSC) in the bone marrow (BM). The progenitor cells affect each other in their differentiation by release of migration factors (i.e. SCF-1) or direct cell adhesion interactions (1, 2). The deregulation of the balance between OBs and OCs can lead to chronic inflammatory diseases like osteoporosis or rheumatoid arthritis (RA) (3). This is reflected in the synovial fluid of RA patients, where OCs are present at pathological levels. An efficient treatment of these pathologies is the exposure to low doses of ionizing irradiation, either photons or exposure to α- particles in radon galleries.
Therefore we want to assess whether low dose irradiation influences differentiation or survival of BM progenitor cells. We focused first on OB progenitors as well as differentiated cells.

Materials and Methods

MSC were isolated from BM aspirates of healthy donors via their adherence to plastic and further differentiated into OBs with ß-glycerophosphate and ascorbic acid treatment. The differentiation status was controlled by staining of $Ca2^{+}$ deposits with Alizarin red S (4). Immunofluorescence staining (IF) was performed with DAPI to highlight the nuclei, and antibodies to NF-kB/p65 or to its phosphorylated form to score the presence and activation of NF-κB.

Results and Discussion

First experiments revealed that the characteristic deposition of $Ca2^{+}$ during differentiation of OBs increased not only with increasing cell passage numbers and cultivation time (Fig.1a) but also after X-ray exposure in a dose-dependent manner (0.5 and 2 Gy) (Fig.1b). Furthermore, OBs showed reduced clonogenic survival (26% after exposure to 2Gy X-rays) and were more radiosensitive than other mesenchymal cells, i.e. fibroblasts (5). With further differentiation, osteoblasts became even more radiosensitive (not shown).

Alizarin Red S Assay

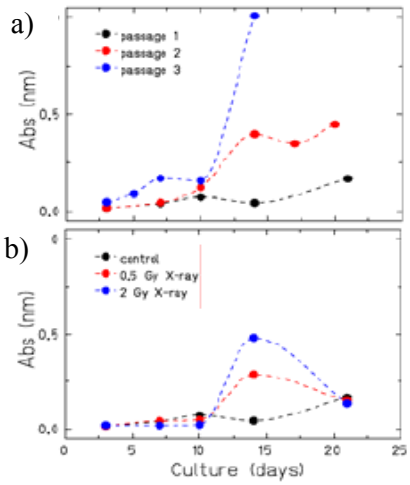

Fig.1: Alizarin Red S absorbance (450 nm) measured in cell extracts of OBs throughout cultivation time (a) and after irradiation with X-ray (0.5 and 2Gy) (b). Increasing absorbance values indicates enhanced Ca^{2+} deposition as result of faster cell differentiation.

The radiation induced enhanced differentiation and reduced survival could potentially impact the balance between OBs and OCs after irradiation. To investigate the involvement of NF-κB, which is an important factor regulating survival and differentiation, we have established the conditions to score its activation by measuring its phosphorylation as well as its nuclear translocation in osteoblasts *in situ* (Fig.2) and *in vitro*.

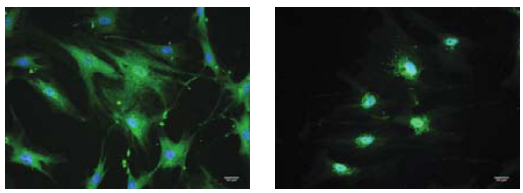

Fig.2: IF staining (green) of NF-κB/p65 (a) and NF-κB/p65phospho (b) in TNF-α stimulated primary osteoblasts. DAPI is used for nuclei staining; Activated NF-κB/p65phospho is located in nucleus of OBs.

References

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