Differentiation of osteoblasts and adipocytes following irradiation*

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Introduction

Chronic inflammatory diseases such as Rheumatoid Arthritis (RA) or Ankylosing Spondylitis are associated with dysregulation of the balance between remodelling and degradation of bones. Chronic inflammatory disorders are treated with anti-inflammatory drugs, but also with ionizing radiation, where joints are locally irradiated with low doses of photons or the patients are exposed in galleries or baths to the α-emitter radon (1). Despite the clinical success, the cellular and molecular mechanisms are largely unknown. As inflammatory processes are closely linked to the regulation of bone metabolism (2) and the differentiation of mesenchymal stem cells (MSC) into osteogenic or adipogenic progenitor cells is linked to bone formation or the production of fat cells (3), we hypothesize that radiation exposure could shift the balance from adipocytes to bone forming osteoblasts (OBs) and thereby counteract the inflammatory processes and bone resorption.

Materials and Methods

OBs were generated from bone marrow aspirates of healthy donors (blood donor service Frankfurt/Main). Differentiation of OBs was initiated by adding β-glycerophosphat and ascorbic acid. Protein expression was analysed by qRT-PCR. Adipocytes were differentiated from commercially available pre-adipocytes (Lonza) by addition of defined adipogenic cocktail, including insulin and 3-isobutyl-1-methylxanthine. Mature adipocytes were identified by Oil Red O staining. Irradiation was performed with X-rays (1Gy/min).

Results

Protein release in osteoblasts following irradiation

The measurement of protein release in OBs following X-ray exposure (Fig.1) has shown that low and moderate dose of ionizing radiation leads to increased protein release of osteocalcin (OCN) (Fig.1), which contributes to bone matrix formation. This results are in line with our previous results (4),where we observed up to 3x fold increased calcium deposition of OBs following exposure to low and moderate doses of X-rays, indicating an accelerated differentiation. However the release of osteoprotegerin (OPG), an inhibitor of osteoclastogenesis, was not affected by irradiation. This indicates that radiation influences rather bone formation than the maturation of osteoclasts via OPG release from irradiated OBs.

Differentiation of Adipocytes

The ongoing experiments include the analysis of gene and protein expression, related to the differentiation of adipocytes and release of pro-inflammatory factors in mature adipocytes after irradiation. The first results do not show significant changes in the differentiation capacity of preadipocytes, assessed by the lipid accumulation (Fig.2). Taken together radiation exposure has an influence on the differentiation progress of osteoblast precursor cells, but not adipocytes precursor cells.

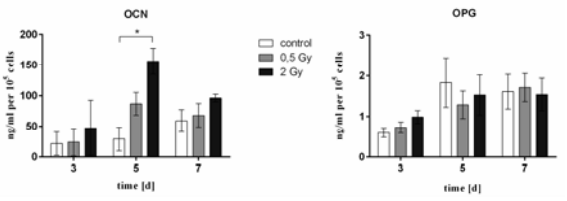

Fig. 1: Protein release in irradiated OBs with X-rays (0.5;2 Gy) after 3, 5 and 7 days. Mean, SEM (N=3). *P<0.05

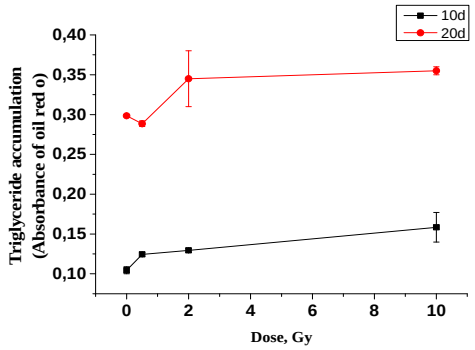

Fig. 2: Effect of X-ray irradiation on adipocyte differentiation. Quantification of stained lipid content in mature adipocytes using Oil Red O extraction and photometric measurement at 540 nm 10/20 days after irradiation. Mean, SEM (N=2)

References

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