X-rays advance osteoblast differentiation probably involving the cholinergic system *in vitro*[*]

G. Thangaraj[1], *C. Fournier*[2], *and P. G. Layer*[1]
[1]Technische Universität Darmstadt, Germany; [2]GSI, Darmstadt, Germany

Introduction

Rheumatoid Arthritis (RA) is an autoimmune disorder and treatment with Radon is believed to suppress the severity of the disease. Thereby, associated with excess pain, cartilage undergoes severe destruction and synovial fluids accumulate in the joints. One of the mechanisms supposedly involved in RA is the *cholinergic anti-inflammatory pathway* (CAIP). Although a number of studies have shown that CAIP is involved in suppressing the inflammation through Vagus nerve stimulation, there are no studies existing that show the relationship between radiation therapy of RA and CAIP. In AP6 of the GREWIS project, we analyse the effects of radiation (X-rays, radon) on the expression of cholinergic components (ACh receptors, particularly the α7-nAChRs, a main player of CAIP, and AChE, ChAT), both *in vivo* and *in vitro* and their roles during radiation therapy.

Material and Methods

11/11.5 day-old embryos from pregnant C57BLC wild type mice were collected. Mesenchymal cells were isolated from limb buds, plated as high density micro-mass cultures and incubated for 2 weeks at 37°C. The micromass cultures were exposed to 0.5, 2 and 4 Gy X-rays initially after 24 hours of culturing and were fixed on 3, 5 and 7 days. Also, human primary osteoblast cells were cultured until passage 4, exposed to above mentioned X-ray doses and collected for mRNA isolation. Alcian blue staining marks cartilage development, while Alizarin red and alkaline phosphatase stainings indicates differentiation of osteoblasts. Cholinesterase enzyme activity was visualized by Karnovsky-Roots staining. cDNA was synthesized and used for PCR analysis.

Results

We previously showed that human osteoblast cells expressed an entire set of cholinergic components. These cells over-expressed acetylcholinesterase (AChE) when exposed to X-rays, which indicates that the cholinergic system might be involved in differentiation and osteogen-esis.

Cultures that were exposed to 2Gy X-rays showed earlier nodule formation and increased enzymatic activity for AChE and alkaline phosphatase suggesting that X-rays might induce an earlier differentiation by involving the cholinergic system.

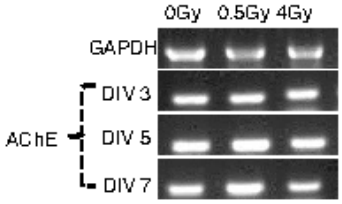

Figure 1: RT-PCR analysis of human osteoblasts. Note an over expression of AChE mRNA, particularly after 0.5Gy X-ray treatment com-pared to that of control (0Gy).

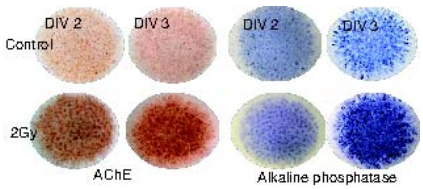

Figure 2: Histological stainings by Karnovsky Roots for AChE (4 on left) and alkaline phosphatase (4 on right) show increased enzyme activity and advanced nodule formation in 2Gy treated cultures.

Since we have focused on the importance of cholinergic system in diseases and treatment, we provide a direct evidence of the involvement of cholinergic system during cartilage differentiation in mouse micromass cultures.

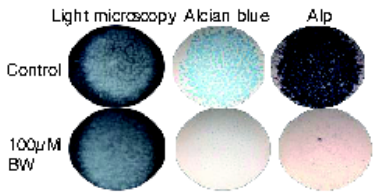

Figure 3: Histological stainings by Karnovsky Roots for AChE (4 on left) and alkaline phosphatase (4 on right) show increased enzyme activity and advanced nodule formation in 2Gy treated cultures.

In future work, the effect of X-rays and radon on TNF-alpha transgenic mice will be investigated and the role of CAIP will be studied.

[*] Funded by BMBF (02NUK017A)

 doi:10.15120/GR-2014-1-BIOPHYSICS-06