

Does Radon act via the cholinergic anti-inflammatory signaling pathway in arthritic mice and mesenchymal stem cells?

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Introduction

Treatment with Radon is believed to suppress the severity of the autoimmune disorder *Rheumatoid Arthritis* (RA). 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). Roles of the cholinergic system in CNS functioning are well established. But also, non-neural, e.g. developmental functions of the cholinergic system are now well accepted. Recently, we have shown improper formation of cartilage in an AChE/BChE double knockout mouse, thus providing evidence for the involvement of cholinesterases in cartilage development (J. Klaczinski, Dissertation 2012). In AP6 of the GREWIS project, we analyse the effects of Radon treatment on the expression of cholinergic components (ACh receptors, particularly the $\alpha 7$ -nAChRs, a main player of CAIP, and AChE, ChAT) during the formation of cartilage, both *in vivo* and *in vitro*.

Materials and Methods

11 day-old pregnant mice and chicken embryos up to stages HH23 are used. Mesenchymal cells were isolated from limb buds of both mouse and chick embryos, plated as high density micro-mass cultures and incubated for 2 weeks at 37°C. Also, human primary osteoblast cells were cultured until passage 4 and collected for mRNA isolation. Alcian blue staining marks cartilage development, while Alizarin red and alkaline phosphatase stainings indicate differentiation of osteoblasts. Cholinesterase enzyme activity was visualized by Karnovsky-Roots staining. cDNA was synthesized and used for PCR analysis.

Results

Human primary osteoblast cells express an entire set of cholinergic components

First results showed that human osteoblast cells expressed cholinesterases (AChE and BChE); they also synthesize ACh and express both muscarinic and nicotinic ACh receptors (Fig. 1), suggesting the involvement of an entire cholinergic system in the differentiation of osteoblasts.



Fig. 1: PCR analyses of human osteoblast cells.

They express all tested cholinergic components.

Micromass cultures as 3D *in vitro*-systems for cartilage formation

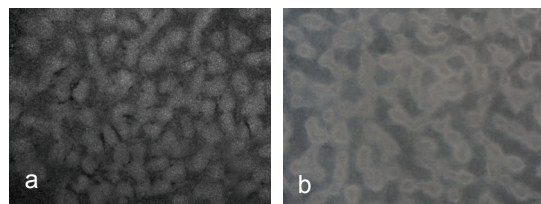


Fig. 2: Light microscopy of micromass cultures. *Chondrocytes condense to form nodules which later differentiate to osteoblasts, both for chick (a) and mouse mesenchymal cells (b). Such cultures will be exposed to radon and to analyze the effects on cartilage formation.*

Formation of cartilage *in vitro*

In mouse micromass cultures, cartilage formation starts as early as div 2, as visualized by Alcian blue staining. It rises until 6 days and drops from 8 days, when osteoblast differentiation takes over.

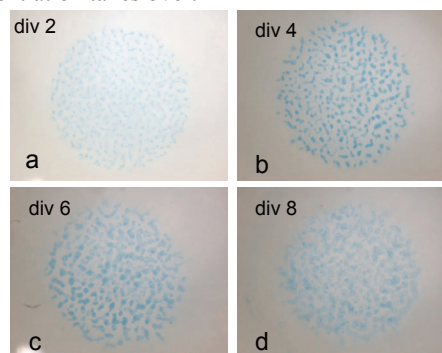


Fig. 3: Cartilage nodules from mouse mesenchymal cells from div2-8, stained with Alcian blue.

Cholinesterase activity during cartilage formation

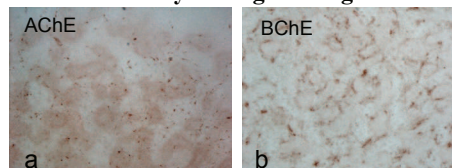


Fig. 4: Note different expression patterns of AChE and BChE in mouse cartilage nodules after 4 div. AChE supposedly supports further osteoblast differentiation.

Future work

Treatment of osteoblasts, MSC-derived chondrogenic cells (to be established) and micromass cultures with cholinesterase and nAChR inhibitors, +/- radon exposure. Correlate changes of cholinergic components with results of other APs (cytokines, NF- κ B, TRP channels).

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