Sympathetic Nerve-Like PC12 Cells Alter Osteoblast Phenotype in the Context of Osteoarthritis

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Statement of Purpose: Osteoarthritis (OA) is the most common form of joint disease and is characterized by continuous degradation of the articular cartilage, inflammation of the surrounding synovium, and alterations in the subchondral bone and its associated vascular and nervous structure[1]. Current treatments for OA cartilage defects generally do not address the inflammatory processes associated with the disease. The pathophysiological mechanism driving OA progression is a circuitous inflammatory cascade involving several tissues and cell types. A deeper understanding of the interplay between these tissues could greatly aid in the discovery and development of effective tissue engineering and regenerative medicine (TERM) based OA therapies. In particular, subchondral bone, a tissue innervated by peripheral sympathetic nerve cells, significantly contributes to the progression of this proinflammatory cascade through biomechanical as well as biochemical factors[2]. In OA, homeostatic bone remodeling processes of subchondral bone are disrupted, resulting in structural abnormalities. Osteoblasts are intimately involved in the remodeling process and also secrete proinflammatory molecules. Therefore, these cells are responsible for the biomechanical and biochemical signals which help propagate the degenerative cascade. Interestingly, the peripheral nervous system contributes to the regulation of bone remodeling and is tightly integrated with the immune system[3]. These characteristics make peripheral sympathetic nerve a potential target for controlling the inflammation created by the altered biomechanical and biochemical signals from subchondral bone. The purpose of this research is to study the influence of sympathetic nerve cell activation on osteoblast phenotype *in vitro* in the context of OA. Results from this research may aid in the design of TERM approaches capable of returning locally activated sympathetic nerve cells to a quiescent state, helping to slow the cycle of degeneration responsible for osteoarthritic disease progression.

Methods: A co-culture model with the sympathetic-nerve-like cell line, PC12, and human osteoblasts (Lonza) encapsulated at $5x10^6$ cells per mL in poly(ethylene glycol) diacrylate (PEGDA) hydrogels was used to study the influence of sympathetic nerve on osteoblast phenotype. To mimic the proinflammatory environment characteristic of OA, 30 nM bradykinin (BK) - a proinflammatory peptide known to activate nerve - was added to media of selected gels. Experimental groups consisted of: 1) unstimulated osteoblasts with and without unstimulated PC12 cells and 2) stimulated osteoblasts with and without stimulated PC12 cells. After 1 week in culture, protein was collected from the osteoblast gels and analyzed for select bone formation (Col 1 and TNAP) and bone resorption ($PTGES_2$, RANKL, and IL-6) markers via western blotting. Data was normalized to levels of β-actin. ANOVA was used for statistical comparison of means.

Results: PC12 cells without BK stimulation significantly upregulated RANKL protein production from osteoblasts (Fig 1B). BK stimulation with PC12 cells also functioned synergistically to significantly increase Col 1, TNAP, $PTGES_2$ and IL-6 from osteoblasts (Fig 1).

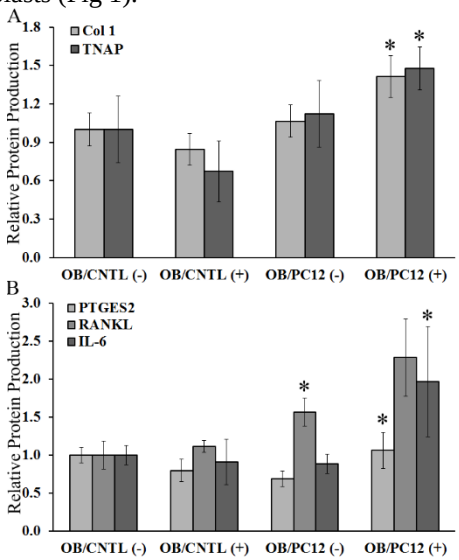

Figure 1. Relative production of various bone (a) formation and (b) resorption proteins from osteoblasts cultured with PC12 cells. Stars above OB/PC12 (-) and OB/PC12 (+) groups indicate a significant difference resulting from PC12 cells without and with bradykinin stimulation, respectively ($p<0.05$).

Conclusions: When stimulated with a proinflammatory peptide that is present in OA, PC12 cells cause an increase in the production of bone formation and bone resorption proteins from osteoblasts. This finding is consistent with the increase in bone remodeling evident in OA. Identifying how sympathetic nerve cells can alter bone metabolism in the context of OA is a first step toward determining potential targets for quieting activated nerve cells. In turn, these targets can be used to design more effective TERM-based OA therapies.

References:

1. Bonnet CS. *Rheumatology* 2005:44.1:7-16
2. Lories RJ. *Nat. Rev. Rheumatol.*2010:7.1:43-49.
3. Elefteriou F. *Arch. Biochem. Biophys.* 2008:473.1:231-236.