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Interstitial Aortic Valve Cells Subpopulations Exhibit Different Calcifying Potential When Exposed to Endotoxin and Phosphate

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Objectives:

To study whether VIC clones isolated from bovine aortic valves (BVIC) exhibit different osteogenic potential in response to endotoxin (LPS) and phosphate (Pi)

Method:

Explants-derived primary BVIC were cloned by limited dilution technique. Cells were treated with combinations of LPS (100 ng/ml) and Pi (2.4 mmol/L). In vitro calcium deposition and alkaline phosphatase activity (ALP) were quantified colorimetrically. BVIC phenotype was established using antibodies anti-: smooth muscle α -actin, type A non-muscle myosin, smooth muscle myosin, CD29, vWF, CD45, osteopontin, osteocalcin. Western blotting and cytofluorimetry analysis were used to establish phenotypic modification in response to the treatments. Selected clonal cells were seeded in type I collagen sponges and treated in vitro as above. Scaffolds calcification was established by von Kossa and Alizarin Red staining.

Results:

LPS increased ALP in uncloned BVIC (day15: LPS 1318 \pm 90, control 243 \pm 80 U/mg protein). Addition of Pi to the cells treated with LPS promoted calcium deposition (day25: LPS 0,07 \pm 0,007, control 0,005 \pm 0,001 mg/mg of protein). We selected four clones displaying different phenotypes. Only Clone1 (fibroblast-like phenotype) showed increase in ALP after LPS treatment. None of the clones calcified after addition of Pi but mineralization was observed in co-culture of Clone1 and Clone4 (SM-like phenotype). LPS treatment of Clone1 inhibited SM markers expression and increased osteocalcin (no modification was observed in Clone4). LPS plus Pi induced calcification of collagen sponges seeded with Clone1.

Conclusions:

Clonal VIC subpopulations harbour different calcifying potential. A specific BVIC subset, endowed with fibroblast-like phenotype, express osteogenic markers and promote calcification in response to LPS and Pi.