Association between **CHONDROCYTE HYPERTROPHY** and **ANGIOGENESIS** of cartilage in **OSTEOARTHRITIS**

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PURPOSE

Chondrocyte hypertrophy is commonly observed in osteoarthritic (OA) cartilage, associated with matrix mineralization and vascularization. As hypertrophic differentiation of chondrocytes is an important feature of osteoarthritis, we developed a model of culture of hypertrophic chondrocytes in order to study the functional consequences of hypertrophic OA chondrocytes. The aim of this study was to investigate the link between hypertrophic differentiation of chondrocytes and angiogenesis in OA in order to demonstrate that OA hypertrophic chondrocytes expressed an angiogenic phenotype and that some specific factors could be implicated in both processes.

METHODS

Articular OA chondrocytes were cultured for 28 days in alginate beads in medium containing 2% Ultroser G (UG) or 10% Fetal Bovine Serum (FBS). DNA was quantified by fluorimetry. The expression of hypertrophy markers genes type X collagen (col10a1), runt-related factor 2 (runx2) and matrix metalloprotease 13 (MMP13) and a screening of angiogenic factors was evaluated by RT-PCR and normalized with HPRT expression. The screening was confirmed by western blot analysis normalized with tubulin-α (55kDa). Alkaline phosphatase (AP) activity and 5'phosphodiesterase activity of NTPPPH were quantified by specific enzymatic methods. Non-hypertrophic and hypertrophic human OA chondrocyte conditioned media were used to perform functional tests with endothelial cells: migration, invasion and wound healing assays.

RESULTS

In alginate beads, OA chondrocytes cultivated 28 days in serum-supplemented medium but not in medium enriched with UG underwent a hypertrophic differentiation process. Indeed, culture of OA chondrocytes in FBS enriched medium was characterized by significant increased expression of hypertrophic markers (col10a1: $p<0.05$; runx2: $p<0.01$; MMP13: $p<0.001$) (Figure 1A) and activity of mineralization enzymes (PA: $p<0.001$; NTPPPH: $p<0.001$) (Figure 1B) with time of culture.

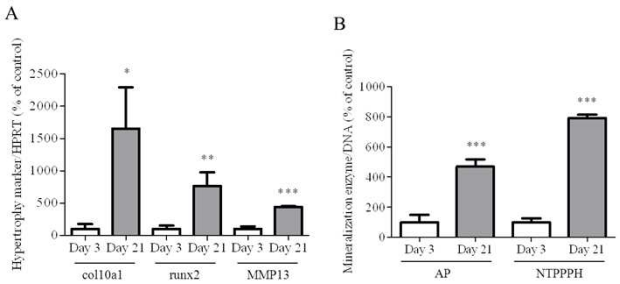

Figure 1 : OA chondrocytes cultivated in alginate beads for 28 days in serum-enriched medium undergo a hypertrophic differentiation process characterized by a significant increase of hypertrophy markers after 21 days of culture

Functional angiogenesis assays showed that hypertrophic chondrocytes positively influenced migration ($p<0.0001$) (Figure 2A), invasion ($p<0.0001$) (Figure 2B) and wound healing ($p=0.0005$) (Figure 2C and D) by endothelial cells.

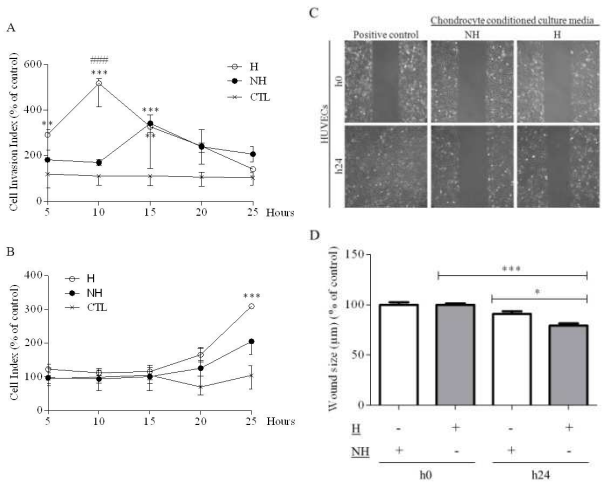

Figure 2 : Hypertrophic chondrocytes positively influence migration and invasion of endothelial cells, two essential steps in the process of angiogenesis

Among the screened angiogenic factors expressed by OA chondrocytes, bone sialoprotein (BSP) expression (Figure3A) and production (Figure 3B) (65 kDa) were highly upregulated in hypertrophic chondrocytes ($p<0.05$).

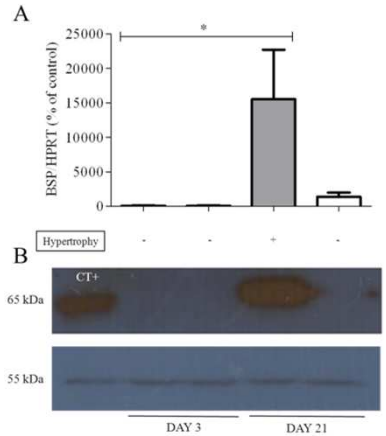

Figure 3 : BSP expression and production are highly upregulated in hypertrophic chondrocytes

CONCLUSIONS

Our culture model allowed to mimic hypertrophic differentiation of chondrocytes and to investigate the relationship between this process and functional invasion and migration of endothelial cells, two functional steps in the process of angiogenesis. The results obtained in this study highlighted BSP as a specific factor that could be implicated in hypertrophic differentiation of chondrocytes and cartilage angiogenesis.

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