BONE SIALOPROTEIN :
A potential key mediator of the angiogenic activity of hypertrophic osteoarthritic chondrocytes

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PURPOSE

Chondrocyte hypertrophy in osteoarthritis (OA) is associated with extracellular matrix mineralization and articular cartilage angiogenesis. In this context, we previously developed a culture model for studying hypertrophic differentiation of OA chondrocytes. Using this model, we have investigated 1) the impact of hypertrophic differentiation on the chondrocytes capacity to promote vascularization 2) the production of a factor associated with angiogenesis by hypertrophic chondrocytes 3) the regulation pathway of this factor in OA.
More precisely, we have investigated the effects of hypertrophic differentiation on endothelial cells invasion and migration and Bone Sialoprotein (BSP) synthesis and regulation.

METHODS

OA chondrocytes cultured in alginate beads in a medium containing 10% foetal bovine serum (FBS) for 28 days progressively undergo a hypertrophic differentiation, whereas in medium containing 2% Ultroser G (UG) they don't differentiate (control). In these conditions, chondrocytes were then cultured without or with IL-1β (170 pg/ml) or TNFα (25 ng/ml) for 7 (D7), 21 (D21) or 28 (D28) days. Endothelial cells (Huvecs) invasion and migration were followed in real-time using the xCELLigence system under the influence of non hypertrophic (FBS D3) or hypertrophic (FBS D21) chondrocytes conditioned culture media. Huvecs migration was also assessed using a wound healing assay. BSP gene and protein expression were respectively evaluated by RT-PCR and western blot at day 3 (D3) and day 21. Finally, we have studied the effect of increased concentrations of recombinant BSP (25, 100 and 400 ng/ml) on the production of interleukin-8 (IL8) and thrombospondin-1 (TSP1) by OA chondrocytes.

RESULTS

Culture media conditioned by hypertrophic (FBS D21), but not by non-hypertrophic chondrocytes (FBS D3), induced an early and significant endothelial cell invasion (Figure 1A) and late migration (Figure 1B) (**: p<0.001). The wound healing assay showed a clear decrease of the wound size with FBS D21 conditioned culture medium compared to FBS D3 culture medium (Figure 1C-D). This finding confirmed that hypertrophic chondrocytes promote endothelial cell migration.

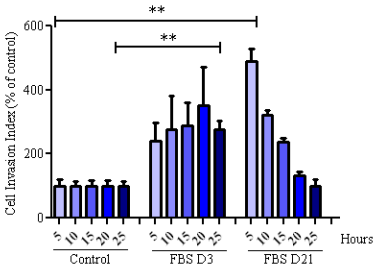

Fig.1A

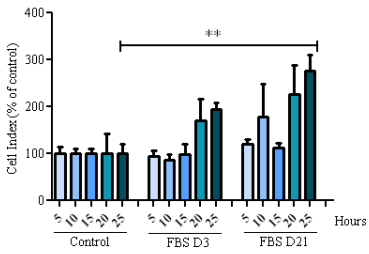

Fig.1B

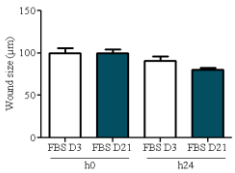

Fig.1C

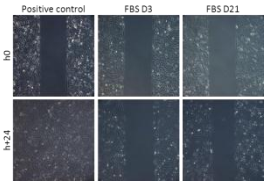

Fig.1D

In FBS, but not in UG, BSP gene expression increased significantly (*: p<0.05) with time of culture (Figure 2A). The protein was detected only in proteic extracts of hypertrophic chondrocytes (Figure 2B). The expression of BSP was correlated to those of specific markers of hypertrophy (data not shown, OARSI 2011).

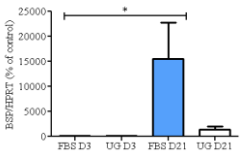

Fig.2A

Fig.2B

In comparison to the control condition (FBS), IL1β and TNFα clearly inhibited BSP gene expression whatever the culture duration (Figure 3). Preliminary results showed that BSP increased IL-8 (r=0.58, p<0.01) but decreased TSP1 expression (r=0.95, p<0.001) by OA chondrocytes in a dose-dependant manner (data not shown).

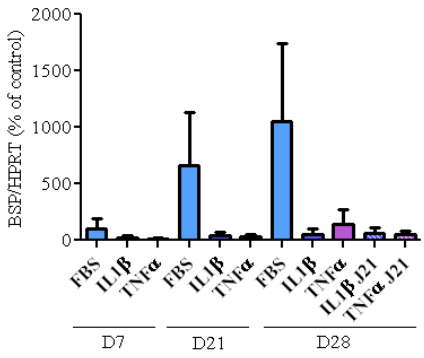

Fig.3

CONCLUSION

Hypertrophic chondrocytes conditioned media stimulate migration and invasion of endothelial cells indicating that hypertrophic chondrocytes express a pro-angiogenic phenotype. BSP expression is associated with hypertrophic differentiation of OA chondrocytes suggesting that it could be a key mediator of the hypertrophic chondrocytes-induced angiogenesis. This hypothesis coud be supported by the inhibiting effect of BSP on TSP-1, a anti-angiogenic factor. To control or reverse chondrocyte hypertrophic differentiation is a promising way for the treatment of OA. In this context, BSP is a potential target for OA drug treatments.