Supplementary data

FGF2 induces RANKL gene expression as well as IL1β regulated MHC class II in bone marrow derived human mesenchymal progenitor stromal cells.

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MATERIALS and METHODS

Cells, growth factors and cytokines

Human BM-MSC were obtained from healthy donors and in vitro expanded as previously described in complete (10% FBS) αMEM (CTR medium). FGF2 (5ng/ml) (R&D) was added when stated. Articular chondrocytes were obtained and cultured as previously described. 1,2

Early passage fibroblasts were obtained from a lung biopsy and expanded in DMEM medium supplemented with10% FBS. IL1β (Sigma-Aldrich), IFNγ (R&D), and the inhibitor SB203580 (Cell Signaling Technology) were used as described in figure legends.

RT-PCR

These were performed as previously described from cDNA isolated from BM-MSC expanded in various conditions as described in the figure legends. RT-PCR primers and probes for the human genes β-actin and CIITA, were from: Assays on Demand, Gene Expression products (Applied Biosystems).

Flow Cytometry (FACS)

This was performed as previously described. 2 All antibodies were from BD (Allschwil, Switzerland).
RESULTS

IL1β lowers the HLA-DR expression induced by IFNγ in articular chondrocytes and lung fibroblasts

The induction of HLA-DR by FGF2 occurs only in proliferating progenitor hMSC, whether from BM or adipose tissue while that by IFNγ is ubiquitous in undifferentiated and differentiated cells. We tested in cultured lung fibroblasts (1 donor) and articular chondrocytes (from 3 donors) whether IL1β did affect IFNγ induced HLA-DR expression. IL1β, using the same experimental conditions as in hBM-MSC, significantly reduced HLA-DR in each cell type as illustrated in figure 1A and 1B.

Cellular p38MAPK controls HLA-DR induced by either FGF2 or IFNγ at a post-CIITA transcriptional level.

The phosphorylation of p38MAPK by IL1β prompted us to investigate whether p38MAPK also affects the expression of CIITA and HLA-DR induced by FGF2 or IFNγ. As neither FGF2 nor IFNγ activates p38MAPK, SB203580 will inhibit only baseline/constitutive p38MAPK. In both FGF2 expanded BM-MSC and CTR BM-MSC exposed to IFNγ, the addition of SB203580 lowers HLA-DR surface expression, measured by FACS (fig 2B and 2C) but does not lower CIITA mRNA measured by RT-PCR (fig 2A), suggesting a common CIITA post-transcriptional control by p38MAPK on HLA-DR expression. The addition of IL1β to SB203580 further lowered HLA-DR only in FGF2 BM-MSC. In the experimental conditions used, the SB203580 inhibition of P-p38MAPK is not relieved by the increased level of P-p38MAPK induced by IL1β in both FGF2 and CTR BM-MSC.

LITERATURE CITED


FIGURE LEGENDS

**Figure 1.** Effect of IL1β(IL1)(1-5ng/ml) on HLA-DR, measured by FACS analysis, induced by IFNγ(IFN)(5ng/ml) in lung fibroblasts(LF)(A) and articular chondrocytes(CHO)(B). CTR: control cells. Filled grey histograms are control IgG.

**Figure 2.** (A):RT-PCR analysis of CIITA expression in BM-MSC (3 donors) induced by FGF2 or IFN ± SB203580. HLA-DR expression in hBM-MSC is p38MAPK dependent. FACS analysis of HLA-DR expression in: (B)FGF2 BM-MSC(FGF) ± IL1β(IL1)± SB203580; (C)CTR-BM-MSC ± IL1β(IL1) ± IFNγ(IFN) ± SB203580. Filled grey histograms are control IgG.