New insights into endothelial-to-mesenchymal transition

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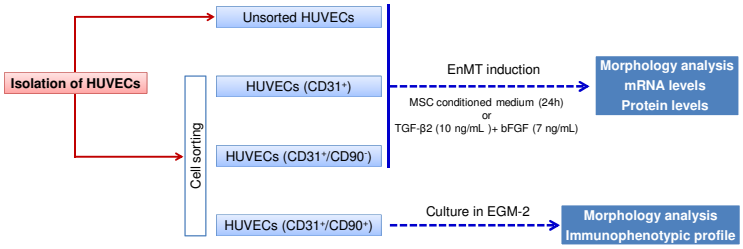
ABSTRACT

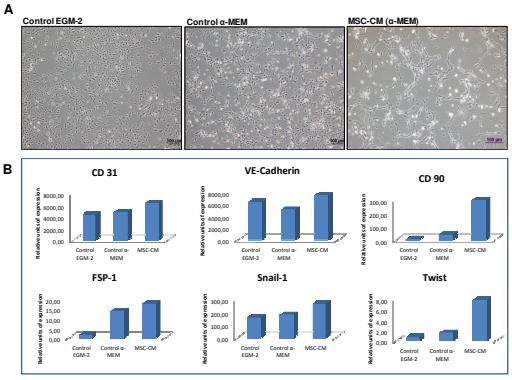
Endothelial mesenchymal transition (EnMT) is a complex event categorized as a form of epithelial mesenchymal transition (EMT) where endothelial cells (EC) lose their adherence junctions and specific markers and acquire mesenchymal/myofibroblast phenotype together with invasiveness and migratory properties. During embryonic development, EnMT participates in heart development, however many studies also associate EnMT to pathological conditions such as cancer and fibrosis. Mechanisms that trigger EnMT are not totally elucidated, together with the role of this process in adult tissues. Assuming the close interaction between the vascular endothelium and stromal cells, especially mesenchymal cells (MSCs), our hypothesis claims that as EMT and EnMT share the same mechanisms of action. So, we asked if human umbilical vein endothelial cells (HUVECs) are able to convert into mesenchymal phenotype *in vitro*.

EXPERIMENTAL DESIGN



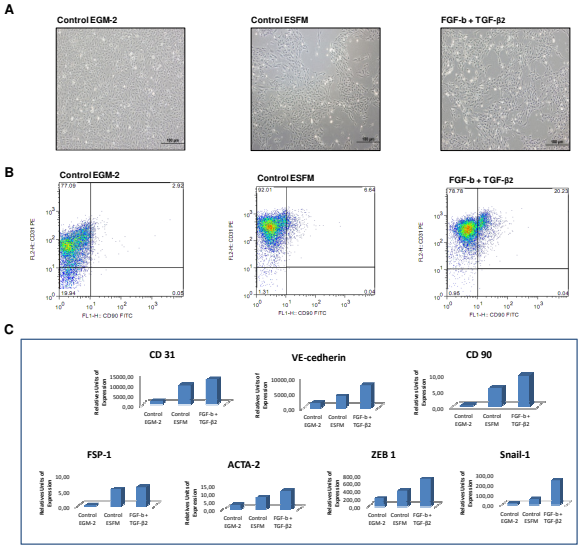
RESULTS

• MSCs' secretome increases the expression of EndMT-related markers in unsorted HUVEC population



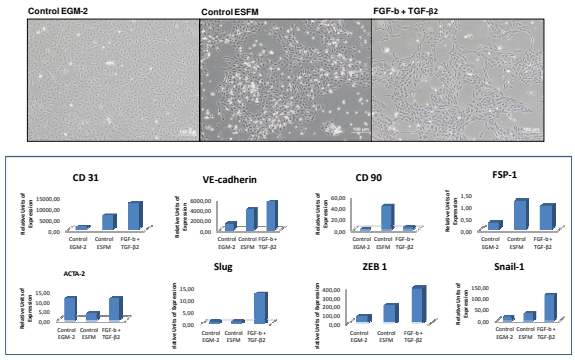
Unsorted HUVECs (77% CD31) acquired a mesenchymal-like morphology upon culture in MSC-conditioned medium **(A)**. Such phenotypic transition was accompanied by the up-regulation of the mesenchymal markers CD90 and FSP-1 and of the EMT-related transcription factors Snail-1 and Twist **(B)**.

• TGF-β2 and bFGF induce morphological and gene expression changes consistent with EnMT in unsorted HUVECs



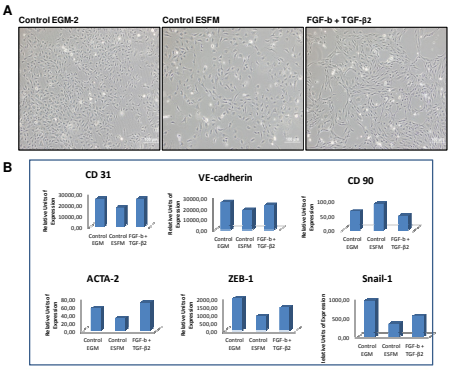
Unsorted HUVECs (77% CD31) acquired a mesenchymal-like morphology after a 3-day incubation in recombinant bFGF and TGF-β2 **(A)**. In addition, we observed an enrichment of the CD31+/CD90+ population by flow cytometry **(B)** which was accompanied by upregulation of the mesenchymal markers CD90, FSP-1, ACTA-2, and the transcription factors ZEB-1 and Snail-1 **(C)**. Interestingly, the acquisition of mesenchymal markers was not followed by repression of the endothelial markers CD31 and VE-cadherin **(C)**.

• Incubation in TGF-β2 + bFGF increases the expression of EMT-related transcription factors in CD31+ sorted HUVECs



After CD31+ separation by sorting, TGF-β2 + bFGF induction triggered a morphologic change to a fibroblastic shape in these cells **(A)**. This was accompanied by the up-regulation of the EMT-related transcription factors Slug, ZEB-1 and Snail-1 **(B)**.

• CD31+/CD90- HUVECs do not undergo EnMT

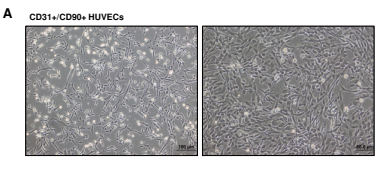


C

	% CD31+	% CD 90+	% CD31+/90+
Control EGM-2	90	1.0	0.9
Control ESFM	81	0.7	0.6
FGF-b + TGF-β2	96	2.3	2.5

CD31+/CD90- HUVECs failed to undergo EnMT after incubation in TGF-b2 + bFGF. Insignificant morphological changes were observed **(A)**, no changes of gene expression profile **(B)** and in the frequency CD90+ cells **(C)**.

• CD31+/CD90+ HUVECs spontaneously display a mesenchymal-like phenotype *in vitro*



B

Antigen	CD31+/CD90+	CD31+/CD90-
CD13	99,58%	99,54%
CD14	0,00%	0,91%
CD29	99,64%	99,15%
CD31	1,33%	94,13%
CD34	2,03%	9,26%
CD44	79,35%	74,86%
CD45	0,13%	0,04%
CD45e	83,70%	97,11%
CD73	96,08%	82,25%
CD90	87,47%	3,65%
CD105	73,91%	99,12%
CD146	69,60%	92,25%
MHC-I	89,33%	91,60%
MHC-II	1,46%	0,67%
VE-cadherin	0,81%	82,50%

After selection, CD31+/CD90+ HUVECs displayed and evident mesenchymal-like morphology **(A)** and display a immunophenotypic profile similar to that of MSCs **(B)**, even when cultured in EGM-2, the medium used for expansion of endothelial cells.