GENE EXPRESSION OF THE WNT SIGNALING PATHWAY IN MESENCHYMAL STROMAL CELLS FROM ACUTE MYELOID LEUKEMIA PATIENTS

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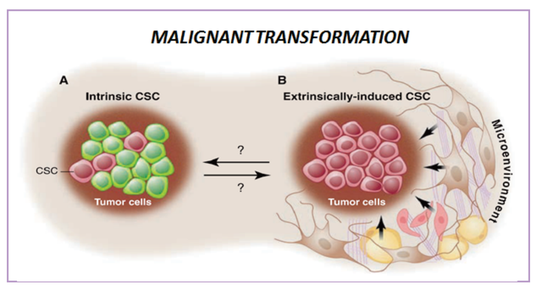
INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematological disease characterized by proliferation and accumulation of myeloid precursors in the bone marrow, decrease in apoptosis level and differentiation arrest of these cells. Although there are several studies in the area, events related to the beginning of the disease as well as its progression are still unknown. It is believed that malignant transformation in normal Hematopoietic Stem Cells (HSC) can give rise to a Leukemic Stem Cell (LSC) and this transformation could be related to changes in Mesenchymal stromal cells (hMSC) signaling. Previous studies showed that mesenchymal stromal cells from Acute Myeloid Leukemia patients (hMSC-AML) have a common molecular signature, different from healthy donors' hMSCs (hMSC-HD) and these differentially expressed genes could be related to malignant transformation. Among the 55 differentially expressed genes, *BMP4* has its expression decreased in hMSC-AML, and this decrease could be regulated by the Wnt pathway.

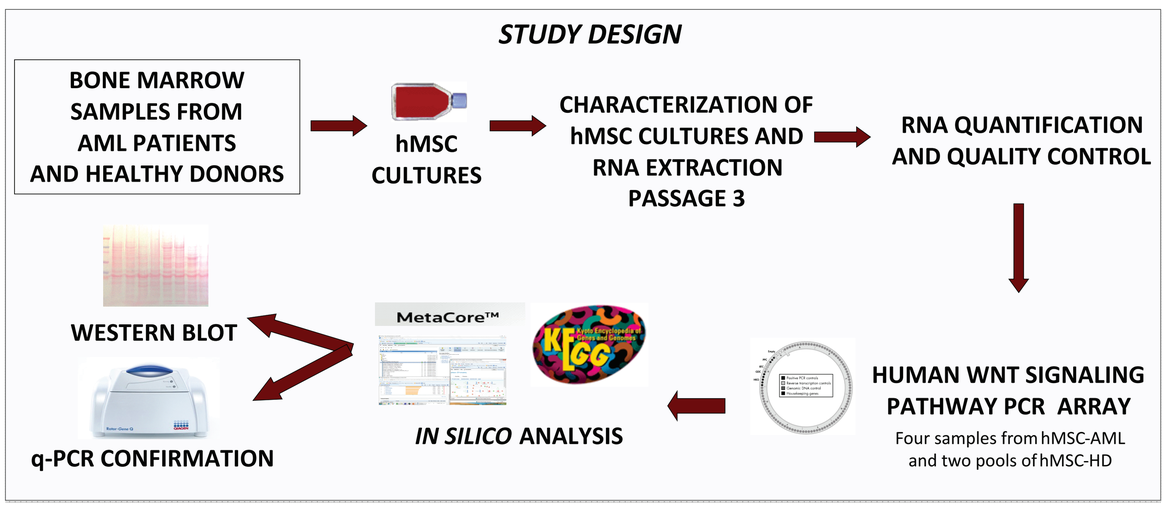


OBJECTIVE

In this context, the aim of this work was to evaluate the gene expression profile of the Wnt pathway in hMSC-AML and hMSC-HD, to verify if this pathway could regulate BMP4 gene.

METHODOLOGY

For this purpose, the Mesenchymal Stromal Cells (hMSC) obtained from both bone marrow patients diagnosed with AML (without any treatment) and healthy donors (HD) were characterized in accordance with the minimum criteria established by the International Society for Cellular Therapy (ISCT). To evaluate the gene expression of 84 genes related to Wnt pathway, we performed PCR Array assay (Human WNT Signaling Pathway RT2 ProfilerTM PCR Array - Qiagen). To confirm the PCR array results, real-time PCR methodology (RT-qPCR) and Western Blot were applied.



RESULTS

hMSC cultures characterization

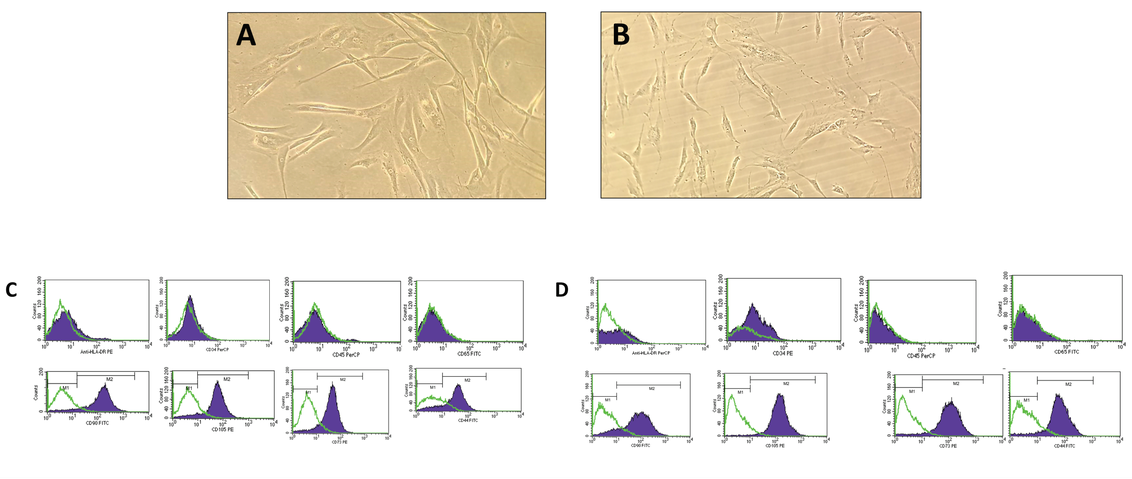


Figure 1: Characterization of hMSC cultures according to ISCT. (A) and (B) Undifferentiated hMSCs from healthy donors and AML patients, respectively (200x magnification). Immunophenotype profile from (C) hMSC-HD and (D) hMSC-AML patients. The cultures were able to express CD90, CD105, CD73 and CD44, in the absence of lineage commitment markers such as CD45, CD34, CD65 and HLA-DR.

Differentially expressed genes from PCR Array assay

Gene Symbol	Fold Change	Gene Symbol	Fold Change
WNT7B	-23,75	PRICKLE1	1,52
WNT11	-3,40	WNT10A	1,56
WIF1	-2,99	BCL9	1,57
CXXC4	-2,44	FZD3	1,64
TCF7	-2,29	KREMEN1	1,76
PORCN	-2,05	VANGL2	1,85
LEF1	-1,86	FRZB	2,39
WNT16	-1,83	FZD1	2,42
WNT5B	-1,73	MMP7	3,11
PITX2	-1,61	SFRP1	3,20
RHOA	-1,57	FZD9	3,56
TCF7L1	-1,56	SFRP4	3,61
PPARD	-1,54	NKD1	9,56

Figure 2: List of the 26 differentially expressed genes when compared hMSC-AML cultures and hMSC-HD, identified by PCR Array assay (Human WNT Signaling Pathway). The fold change 1,5 was used as a criterion to define differentially expressed genes.

RT-qPCR Confirmation

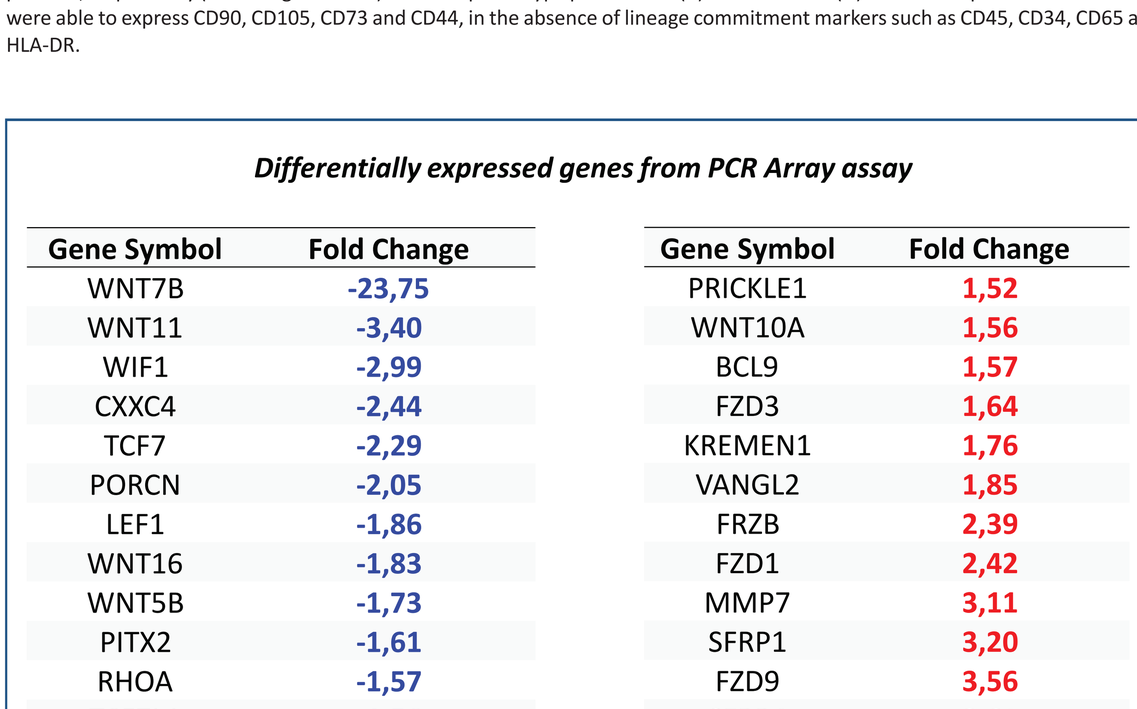


Figure 3: RT-qPCR to validate the PCR array results. To confirm the results obtained in PCR array, RT-qPCR was used to analyze some differentially expressed genes using a larger number of patient samples to determine changes in mRNA expression levels after normalization to B2M and GAPDH (29 hMSC-AML cultures and 21 hMSC-HD cultures). RT-qPCR analysis for TCF7, LEF and PORCN (downregulate in AML patients) and PRICKLE and KREMEN1 (overexpressed in AML patients) confirmed the PCR array assay. *p<0,05 / **p<0,01 / ****p< 0,0001

Western Blot analysis

Figure 4: Western blot analysis of differentially expressed protein found in our study. Total protein extract (25 µg) from hMSC-AML (n = 4) and hMSC-HD (n = 4) were separated by SDS-PAGE and probed with specific antibody (LEF1).

In silico analysis

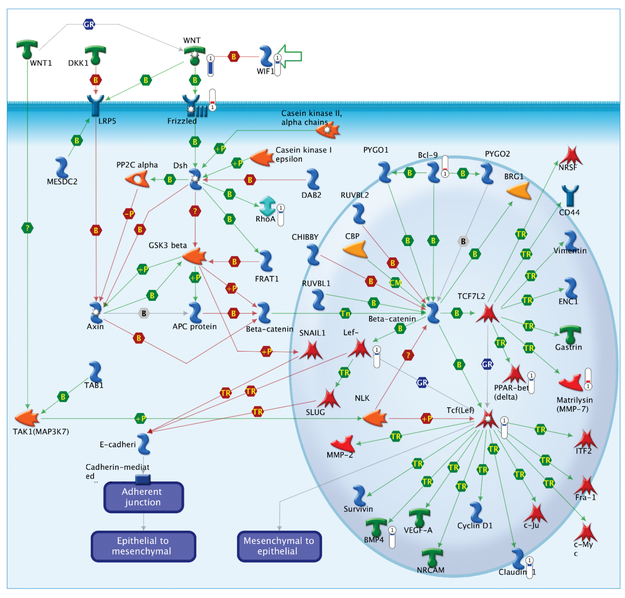


Figure 5: In silico analysis using software MetaCore™ of differentially expressed genes in hMSCs-LMA involved in Wnt signaling pathway.

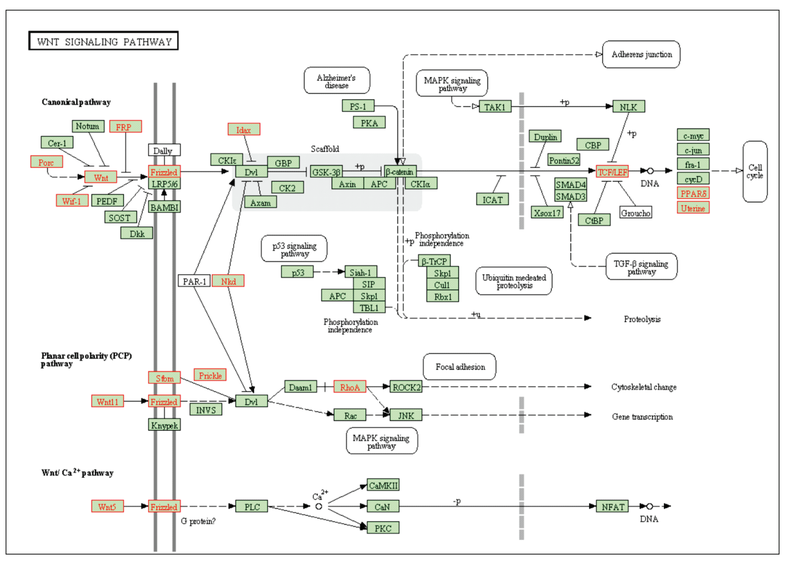


Figure 6: A KEGG diagram of the canonical Wnt/β-catenin pathway: Boxes in green indicate proteins relevant to Wnt signaling. Red boxes indicate changes in hMSC-AML gene expression.

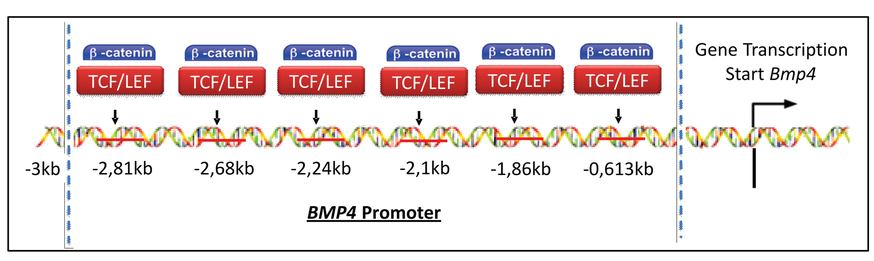


Figure 7: *In silico* analysis of 3kb promoter of the *Bmp4*: Six *Tcf/Lef* binding sites were identified in the 3kb promoter region of the *Bmp4* gene (*Tcf/Lef* Binding sites: 5'-CTTTAG-3' ou 5'CTTTGA3')

CONCLUSIONS

Altogether, our results suggest that the WNT signaling pathway is changed in hMSC-AML from AML patients independent of subtypes and this pathway regulation could be related to the decrease of BMP4 in hMSC-AML.

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