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

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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, apoptosis reduction and differentiation arrest. Although several studies in the area, events related to the beginning of 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 hMSC from Acute Myeloid Leukemia patients (hMSC-AML) have a common molecular signature, compared to healthy donors' (hMSC-HD) and these differentially expressed genes could be related to malignant transformation. Among 55 differentially expressed genes, *BMP4* has its expression decreased in hMSC-AML, and this decrease could be regulated by the Wnt pathway.

Keywords: Acute myeloid leukemia (AML), Mesenchymal stromal cells (MSC), WNT pathway.

OBJECTIVE

In this context, the aim of this work was to evaluate the gene expression profile of Wnt pathway in hMSC-AML.

METHODOLOGY

For this purpose, the 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 confirm the results we performed real-time PCR (RT-qPCR) with a large number of patients and to confirm the protein levels of some differentially expressed genes, Western Blot and flow cytometry assays were performed. The pathway analysis and related processes were obtained using the MetaCore™ and KEGG softwares.

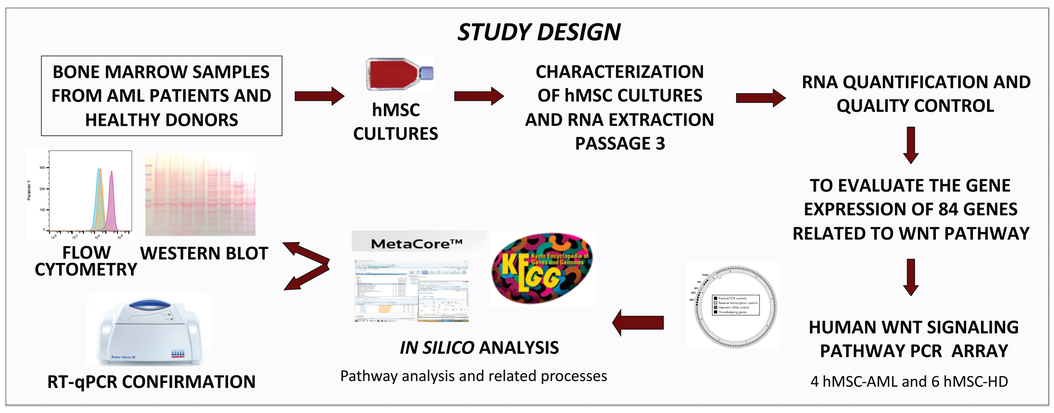

Figure 1. Schematic diagram of the study methodology.

RESULTS

hMSC cultures characterization

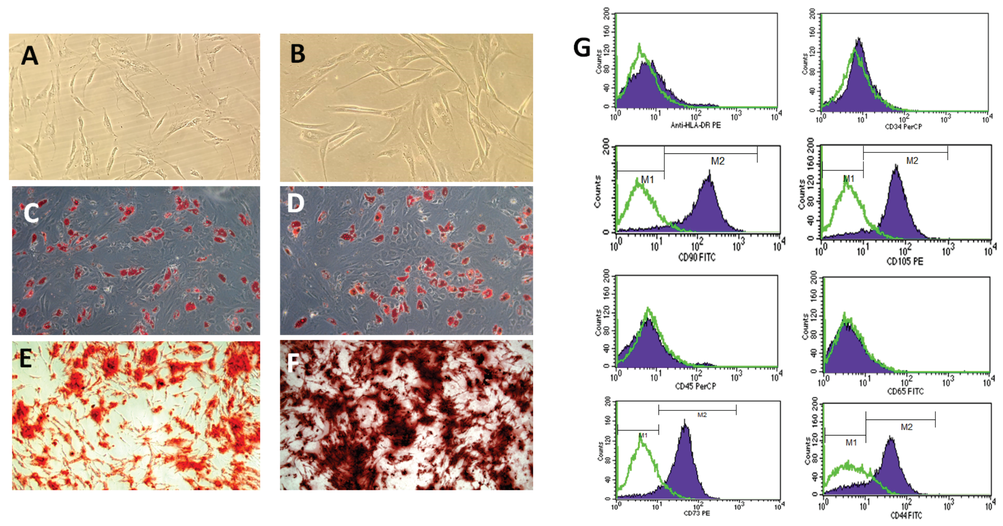

Figure 2: Characterization of hMSC cultures according to ISCT. (A) and (B) – undifferentiated hMSCs from AML patients and Healthy donors (HD), respectively (200x magnification). (C) and (D) – Adipogenic differentiation from AML patients and HD, respectively. Accumulation of neutral lipid vacuoles stained with oil red O indicate differentiation (200x magnification). (E) and (F) – Osteogenic differentiation from AML patients and HD, respectively. Calcium deposition stained with alizarin red indicate differentiation (50x magnification). (G) Immunophenotype profile from hMSC-AML patients and hMSC-HD. 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.

Table 1: List of the 26 differentially expressed genes when compared hMSC-AML cultures and hMSC-HD, identified by PCR Array assay (Human WNT Signaling Pathway). Data were analyzed using GeneGlobal data analysis center (Qiagen) and differentially expressed genes with ≥± 1,5 fold-change was used as a criterion to define overexpression or downregulation.

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

RT-qPCR Confirmation

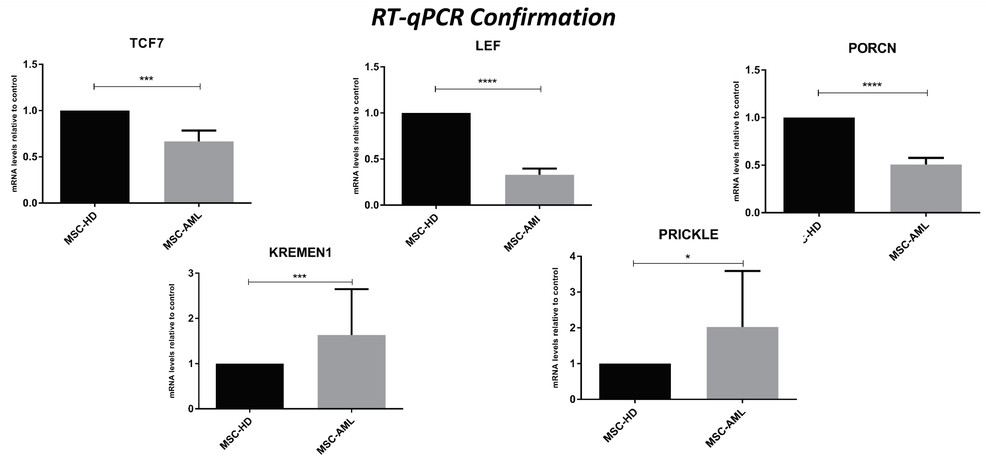

Figure 3: RT-qPCR to validate the PCR array results. To confirm the results obtained in PCR array, RT-qPCR was used to analyze some differentially expressed genes using a larger number of patient samples to determine changes in mRNA expression levels after normalization to *B2M* and *GAPDH* (30 hMSC-AML cultures and 24 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 / ****p< 0,0001

In silico analysis

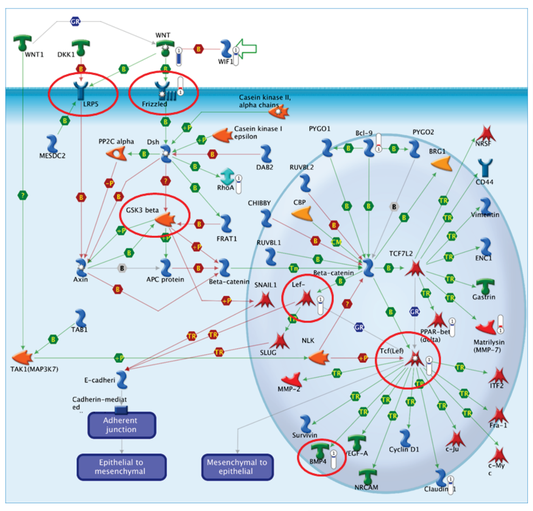

Figure 4: *In silico* analysis using software MetaCore™ of differentially expressed genes in hMSCs-LMA involved in Wnt signaling pathway. *TCF7* and *LEF* are transcription factors, and the decrease of their expression could be related to BMP4 regulation.

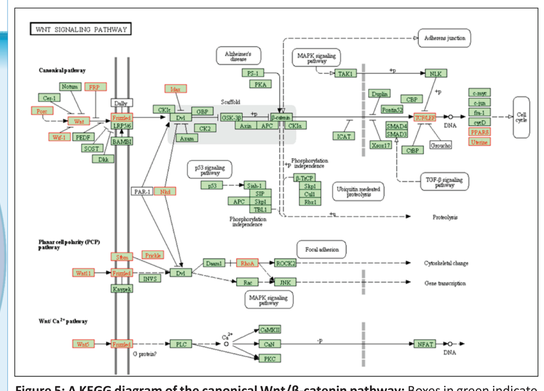

Figure 5: A KEGG diagram of the canonical Wnt/β-catenin pathway: Boxes in green indicate proteins relevant to Wnt signaling. Red boxes indicate changes in hMSC-AML gene expression.

Flow Cytometry analysis

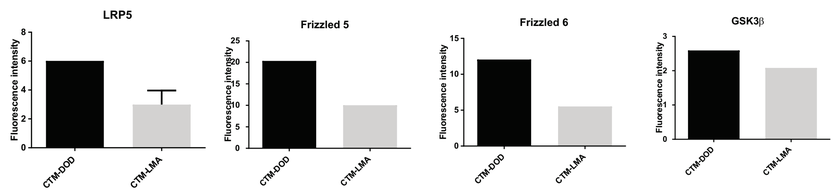

Figure 6: Flow cytometry results show a decrease in the protein level of the membrane receptors of the Wnt pathway (LRP5, Frizzled 5, Frizzled 6) and GSK3β, associated with the stabilization of β-catenin.

Western Blot analysis

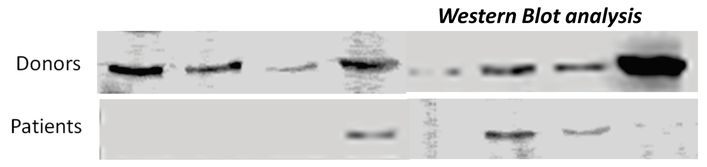

Figure 7: Western blot analysis of differentially expressed protein found in our study. Total protein extract (30 µg) from hMSC-AML (n = 8) and hMSC-HD (n = 8) were separated by SDS-PAGE and probed with specific antibody (LEF1). The results confirmed a decrease of transcription factor in hMSC-AML.

BMP4 promoter region analysis

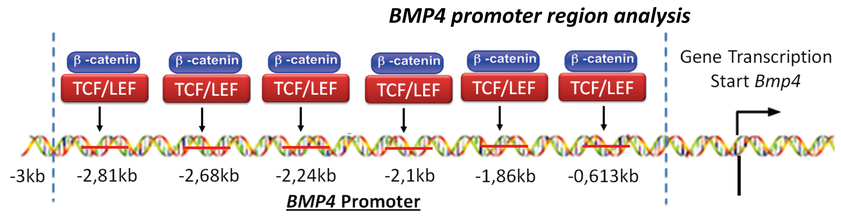

Figure 8: *In silico* analysis of 3kb promoter of the *Bmp4*: Six *Tcf/Lef* binding sites were identified in the 3kb promoter region of the *Bmp4* gene (*Tcf/Lef* Binding sites: 5'-CTTTAG-3' or 5'CTTTGA3'), suggesting that they could regulate BMP4.

CONCLUSIONS

Altogether, these results suggest that the WNT signaling pathway is changed in hMSC-AML and this pathway regulation could be related to the decrease of *BMP4* in hMSC-AML.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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