WNT signaling pathway regulates *Bmp4* expression in Mesenchymal stromal cells from Acute myeloid leukemia patients

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INTRODUCTION

Acute Myeloid Leukemia (AML) is a hematological disease characterized by cellular differentiation arrest, decrease in apoptosis levels, increase in proliferation and accumulation of myeloid precursors in the bone marrow (BM). Although there are several studies in this field, events related to disease initiation and progression remains unknown. The malignant transformation of hematopoietic stem cells (HSC) is thought to generate leukemic stem cells, and this transformation could be related to changes in Mesenchymal stromal cell (hMSC) signaling. A molecular signature from hMSC from AML patients (hMSC-AML) has been proposed by our group, and its gene expression could be related to the leukemic transformation process. We highlight *BMP4,* which has its expression decreased in hMSC-AML and in plasma of AML patients, and this expression could be regulated *in silico* by WNT signaling pathway

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

OBJECTIVE

In this context, the aim of this work was to verify if WNT signaling is capable of regulating *BMP4* gene in hMSCs

METHODOLOGY

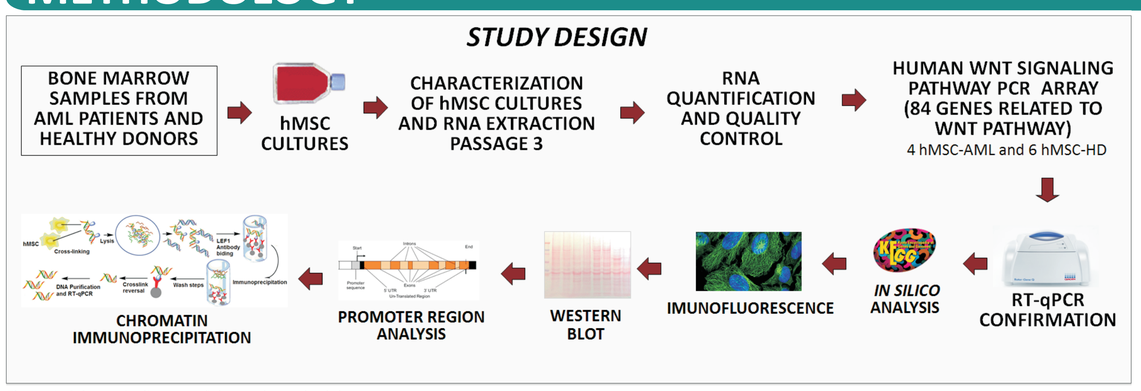

Figure 1. Schematic diagram of the study methodology.

RESULTS

hMSC cultures characterization

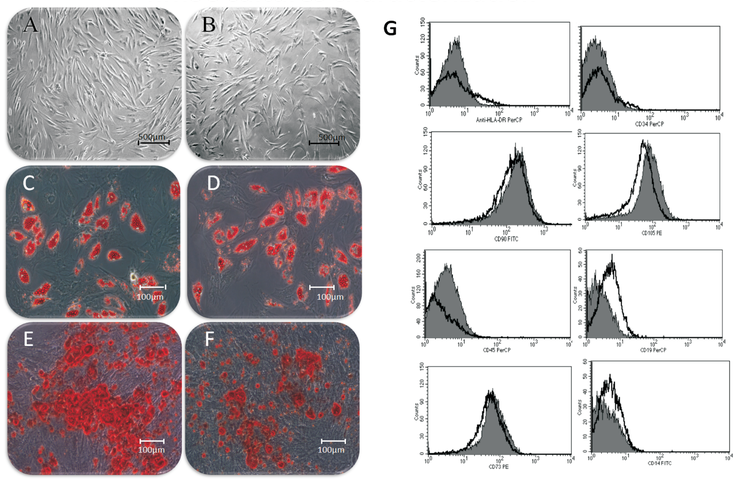

Figure 2: **Characterization of hMSC cultures according to ISCT**. (A) and (B) – undifferentiated hMSCs from AML patients (hMSC-AML) and healthy donors (hMSC-HD), respectively (200x magnification). (C) and (D) – Adipogenic differentiation from hMSC-AML and hMSC-HD, respectively (200x magnification). (E) and (F) – Osteogenic differentiation from hMSC-AML and hMSC-HD, respectively 50x magnification. (G) Immunophenotype profile from hMSC-AML 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.

Differentially expressed genes from PCR Array assay

Gene Symbol	Fold Change
WNT7B	-23,75
WNT11	-3,40
WIF1	-2,99
CXXC4	-2,44
TCF7	-2,29
PORCN	-2,05
LEF1	-1,86
WNT16	-1,83
WNT5B	-1,73
PITX2	-1,61
RHOA	-1,57
TCF7L1	-1,56
PPARD	-1,54

Gene Symbol	Fold Change
PRICKLE1	1,52
WNT10A	1,56
BCL9	1,57
FZD3	1,64
KREMEN1	1,76
VANGL2	1,85
FRZB	2,39
FZD1	2,42
MMP7	3,11
SFRP1	3,20
FZD9	3,56
SFRP4	3,61
NKD1	9,56

Table 1: List of the 26 differentially expressed genes when compared hMSC-AML and hMSC-HD cultures, 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.

RT-qPCR Confirmation

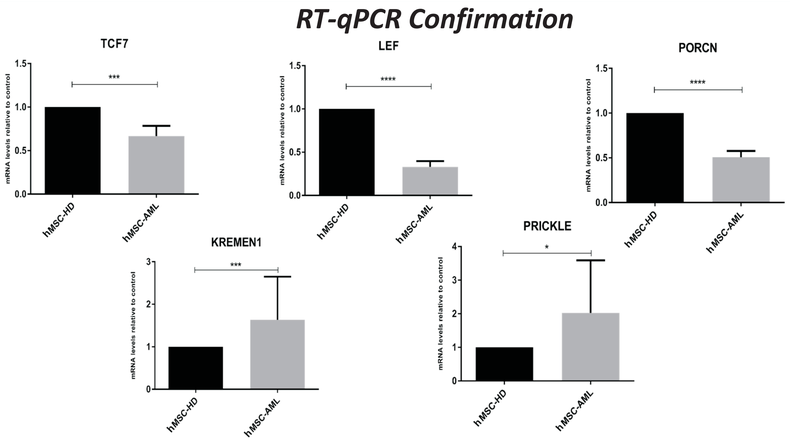

Figure 3: **RT-qPCR to validate the PCR array results.** To confirm the results obtained in PCR array, RT-qPCR was used to analyze some differentially expressed genes using a larger number of samples to determine changes in mRNA expression levels after normalization to *B2M and GAPDH* (30 hMSC-AML cultures 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. The data were expressed as the mean ± SD. *p<0,05 / **p<0,01 / ****p< 0,0001 / ****p< 0,0001

In silico analysis

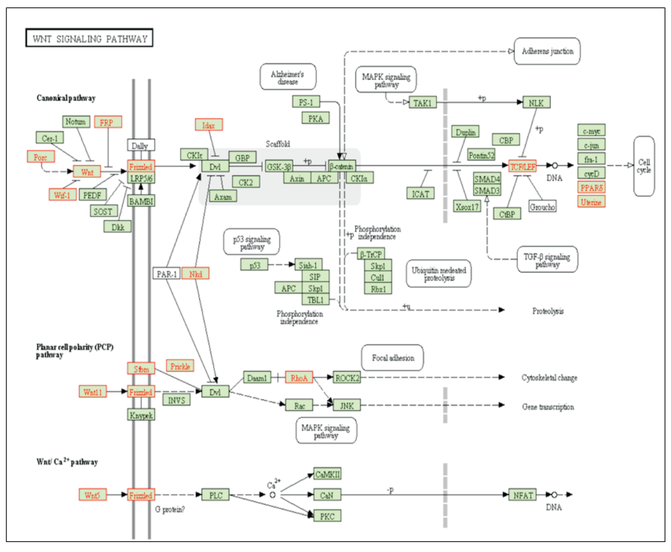

Figure 4: **WNT Signaling Pathway:** Boxes in green indicate proteins relevant to Wnt signaling. Red boxes indicate changes in hMSC-AML gene expression.

β-CATENIN *Immunofluorescence*

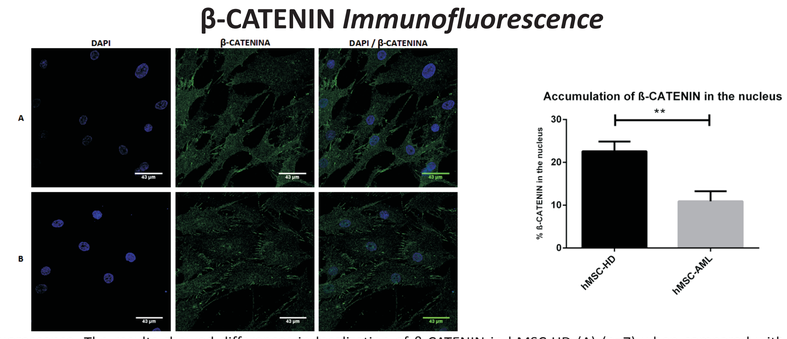

Figure 5: β-CATENIN Immunofluorescence. The results showed differences in localization of β-CATENIN in hMSC-HD (A) (n=7) when compared with hMSC-AML (B) (n=6) (63x magnification). **p<0,01

LEF1 Western Blot analysis

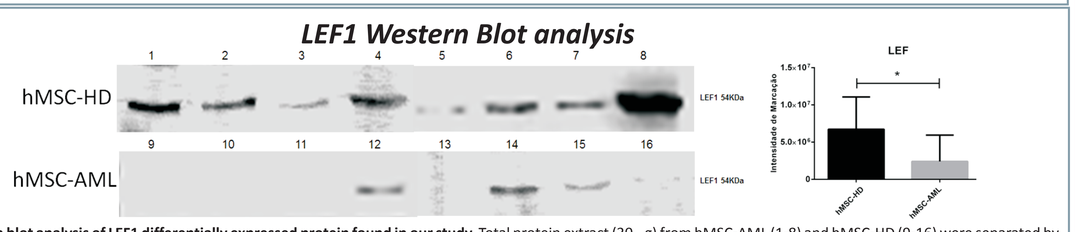

Figure 6: Western blot analysis of LEF1 differentially expressed protein found in our study. Total protein extract (30 g) from hMSC-AML (1-8) and hMSC-HD (9-16) were separated by SDS-PAGE and labelled with specific antibody (LEF1). The results confirmed a decrease of the transcription factor LEF1 in hMSC-AML. The data were expressed as the mean ± SD. *p<0,05

β-CATENIN *and* LEF1 *Immunofluorescence*

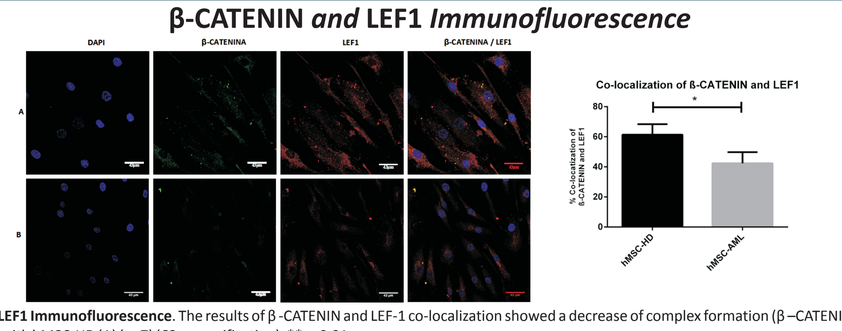

Figure 7: **β-CATENIN and LEF1 Immunofluorescence**. The results of β -CATENIN and LEF-1 co-localization showed a decrease of complex formation (β –CATENIN/TCF-LEF) in hMSC-AML (B) (n=6) when compared with hMSC-HD (A) (n=7) (63x magnification). **p<0,01

BMP4 promoter region analysis

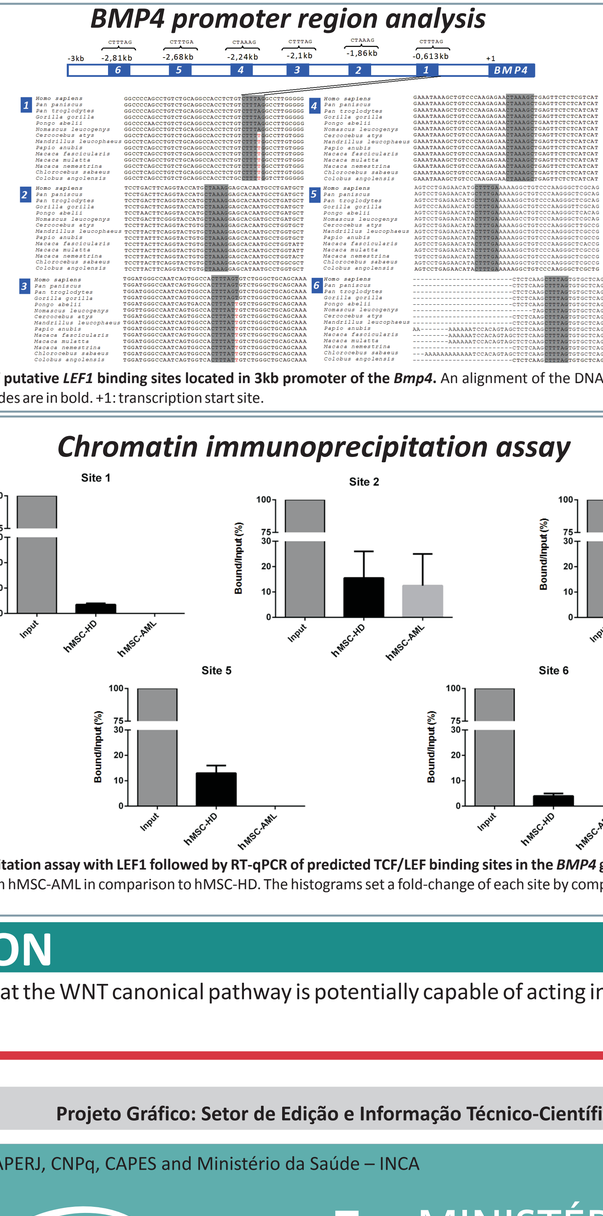

Figure 8: **Representative scheme of putative *LEF1* binding sites located in 3kb promoter of the *Bmp4*.** An alignment of the DNA region showed evolutionarily conservation among mammals species. Identical nucleotides are in bold. +1: transcription start site.

Chromatin immunoprecipitation assay

Figure 9: **Chromatin immunoprecipitation assay with LEF1 followed by RT-qPCR of predicted TCF/LEF binding sites in the *BMP4* gene promoter.** We observed less binding of LEF1 in TCF/LEF consensus binding sites from hMSC-AML in comparison to hMSC-HD. The histograms set a fold-change of each site by comparing the Input control. The data were expressed as the mean ± SD.

CONCLUSION

Altogether, we suggest that the WNT canonical pathway is potentially capable of acting in the regulation of the BMP4 gene in hMSC-AML

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

Financial support: FAPERJ, CNPq, CAPES and Ministério da Saúde – INCA