Gene expression evaluation of WNT signaling components and Polycomb and Thritorax members in resistance models of chronic myeloid leukemia

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INTRODUCTION

Although treatment of chronic myeloid leukemia (CML) has improved since the introduction of tyrosine kinase inhibitors such as imatinib mesylate (IM), cases of resistance have been reported over the years. WNT signaling (canonical and non-canonical pathways) and activation of members of the Polycomb family (PcG) are known to be related to the maintenance of the normal hematopoietic stem cell (HSC) and are deregulated in the leukemic stem cell (LSC), conferring resistance to MI therapy. However, the cross-talk of these pathways and their implication in resistance have not yet been fully elucidated.

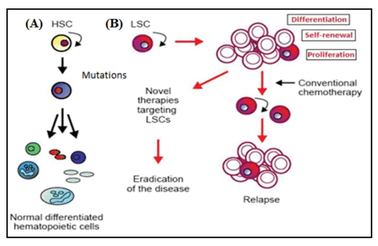


Figure 1. Normal hematopoietic stem cell (HSC) (A) and (B) leukemic stem cell (LSC).

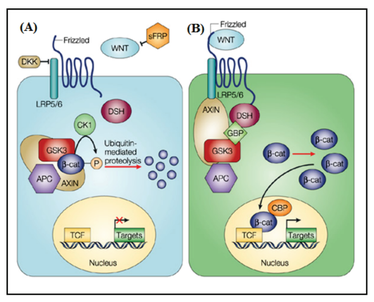


Figure 2. Wnt/β-catenin pathway. (A) Wnt/β-catenin pathway in off-state. (B) WNT ligands bind to a Frizzled receptor (FZD) and the co-receptor (Lrp5/6), the pathway is on-state.

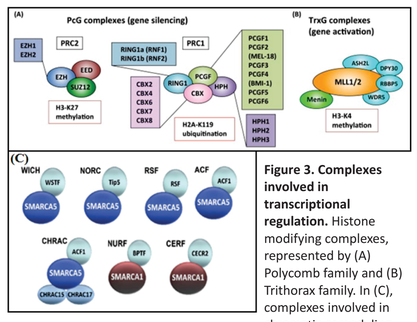
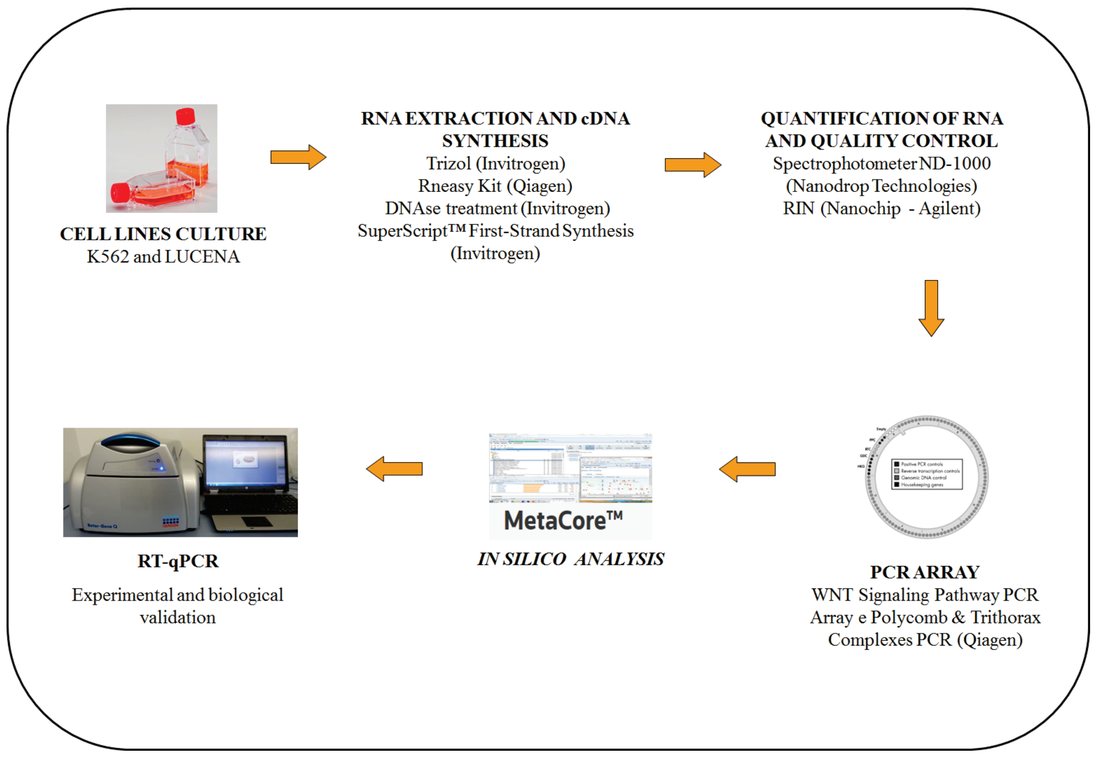


Figure 3. Complexes involved in transcriptional regulation. Histone modifying complexes, represented by (A) Polycomb family and (B) Trithorax family. In (C), complexes involved in chromatin remodeling.

METHODOLOGY



OBJECTIVE

The objective of this work was to evaluate the gene expression of canonical and non canonical members of WNT and the members of PcG and TrxG families in the context of resistance in CML.

RESULTS

Evaluation of gene expression of members of the WNT pathway and the families of Polycomb and Trithorax in CML resistance

IM-resistance in vitro models

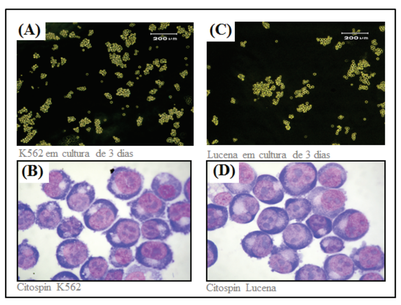


Figure 4. Cell lines K562 (A and B) and Lucena (C and D) in culture.

Differentially expressed genes in IM-resistance

		WNT		Polycomb e Thritorax			
Gene	*Fold-change*	Gene	*Fold-change*	Gene	*Fold-change*	Gene	*Fold-change*
CCND1	287,39	WNT3	-2	ZBTB16	61,31	CTBP2	-2,12
FZD2	98,83	EP300	-2,03	RBP2	19,67	BAP1	-2,18
PRICKLE1	36,68	SFRP1	-2,16	DNMT3B	2,88	RPLP0	-2,18
PPARD	32,6	MMP7	-2,18	CBX2	2,26	KMT2B	-2,37
FZD4	31,49	GAPDH	-2,19			SMARCA4	-2,37
WNT16	8,04	LRP6	-2,37			PHC2	-2,59
TCF7L1	6,48	DVL1	-2,48			PHF1	-2,68
WISP1	5,76	RPLP0	-2,87			RNF2	-2,81
WNT10A	5,16	AXIN1	-3,04			RYBP	-3,04
WNT4	3,86	FZD3	-3,21			TRIM27	-3,1
WNT2B	3,72	SOX17	-3,56			ASXL3	-4,06
NKD1	3,45	DIXDC1	-3,59			SNAI1	-5,82
APC	2,94	DAAM1	-3,74			DNMT3L	-38,64
GSK3A	2,32	WNT3A	-4,48			SMARCA1	-105,6
		RHOU	-6,93				
		WNT8A	-7,85				
		WNT5B	-9,87				
		WNT5A	-14,76				

Figure 5. Differentially expressed genes in WNT signaling and the Polycomb and Thritorax families, obtained from PCR array, when comparing Lucena to K562 in a 2 cut off . The red values represent the overexpressed genes and the blue values, genes with diminished expression.

In silico analysis of interactions and signaling pathways of genes differentially expressed in "in vitro" models of CML resistance

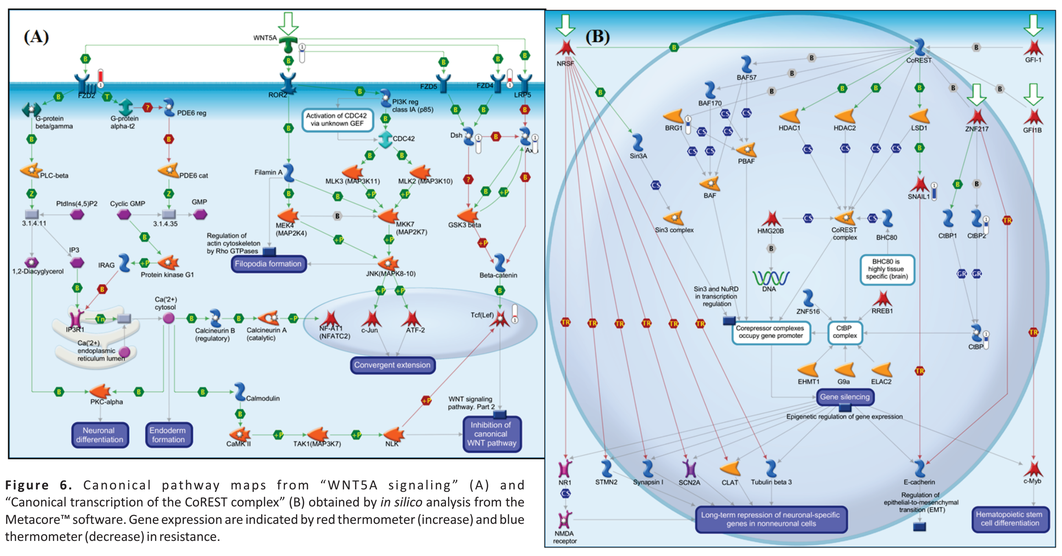


Figure 6. Canonical pathway maps from "WNT5A signaling" (A) and "Canonical transcription of the CoREST complex" (B) obtained by *in silico* analysis from the Metacore™ software. Gene expression are indicated by red thermometer (increase) and blue thermometer (decrease) in resistance.

Experimental and biological validation of the PCR array

Experimental validation of the SMARCA1 and SNAIL1 genes

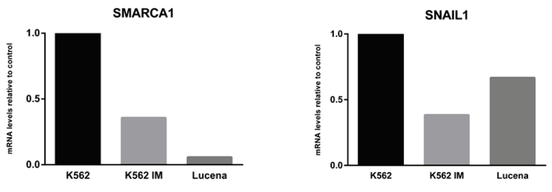


Figure 7. RT-qPCR analysis of mRNA levels of expression of the *SMARCA1* and *SNAIL1* genes in K562, K562 IM and Lucena cell lines. Total RNA was isolated and used in quantitative real-time PCR to determine changes *SMARCA1* expression levels after normalization to β-actin expression.

Biological validation of the SMARCA1 gene in CML patients

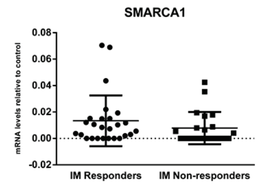


Figure 8. Analysis of mRNA levels of expression of the *SMARCA1 gene* in CML patients responders and non-responders to IM treatment (n=29). Total RNA was isolated and used in quantitative real-time PCR to determine changes *SMARCA1* expression levels after normalization to β-actin expression.

CONCLUSION

The data found in the experimental validation for the *SMARCA1* and *SNAIL1* genes corroborate with the data found in the PCR array. In the biological validation of the *SMARCA1* gene, our results point to a (non-statistical) decrease of this gene in resistant patients, however, patient samples are from the moment they resisted the treatment, wich may indicate its involvement seems to be a consequence and not a cause of resistance, proposing that its regulators and other mechanisms are involved in the acquisition to resistance.

APOIO FINANCEIRO: CNPq, INCT, FAPERJ, Ministério da Saúde.

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