Contribuition of lnc*RNA HOTAIR* in gene expression of chronic myeloid leukemia

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INTRODUCTION

The long non-coding RNAs (lncRNAs) belong to the group of non-coding RNAs involved in epigenetic processes, therefore participating in several levels of gene expression regulation. The long non-coding HOX transcript antisense intergenic RNA (HOTAIR) has been related to the development/resistance of solid tumors; however, in leukemias its role has been poorly explored. In Chronic myelogenous leukemia (CML), so far, there is only one report associating HOTAIR expression to Imatib mesylate resistance. A previous study from our group identified HOTAIR in CML patients´ exosomes (plasma bone marrow). Nevertheless, this expression was not observed in healthy donors exosomes, suggesting its involvement in disease.

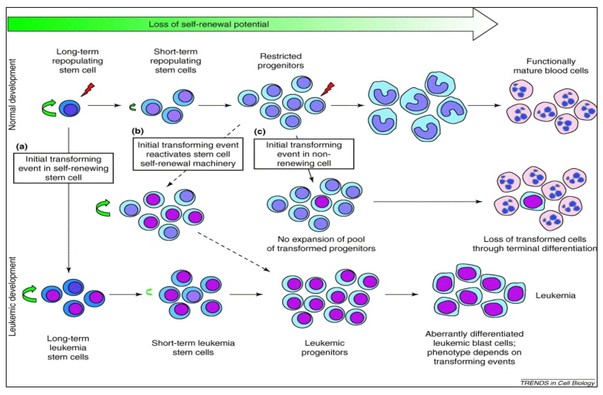


Figure 1. Development of normal hematopoietic stem cell (HSC) and leukemic stem cell (LSC). Mutagenic changes are required to transform stem cells in which the self-renewal machinery is already active (a), as compared with committed progenitors in which self-renewal must be activated ectopically (b). If a committed progenitor acquires a genetic mutation that does not confer increased self-renewal (c), that cell will likely die or undergo terminal differentiation.

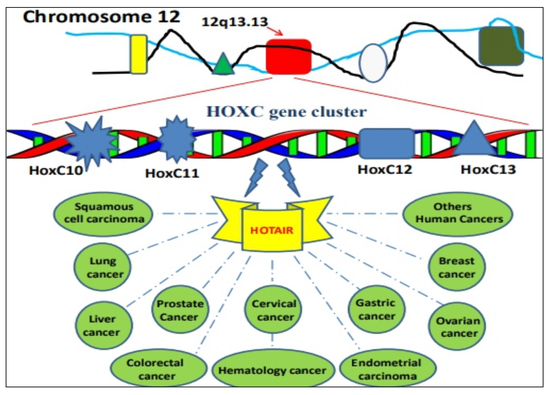


Figure 2. Structure and genomic location of HOTAIR, and association with a variety of human cancers. HOTAIR gene is located HoxC gene cluster on chromosome 12, between HoxC11 and HoxC12. The expression of HOTAIR is association with occurrence and development of human cancers (Tang; Hann, 2018).

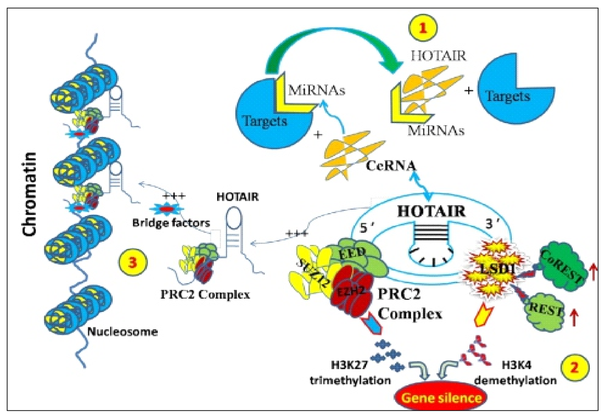


Figure 3. Functions and mechanisms of HOTAIR-mediated gene silencing, regulation of chromatin dynamics, and association between HOTAIR and miRNAs in human cancers. 1) HOTAIR regulates the levels of genes through interaction with miRNAs as a competitive endogenous RNA (ceRNA). 2) HOTAIR binds to polycomb repressive complex 2 (PRC2) and LSD1/CoREST/REST complex, contributing to gene silencing. 3) With the bridge factors, HOTAIR can achieve the regulation of chromatin region and gene expression by guiding the histone modifiers, such as PRC2 (Tang; Hann, 2018).

OBJECTIVE

The aim of this work is to evaluate the contribution of HOTAIR in the CML gene expression.

METHODOLOGY

SAMPLES

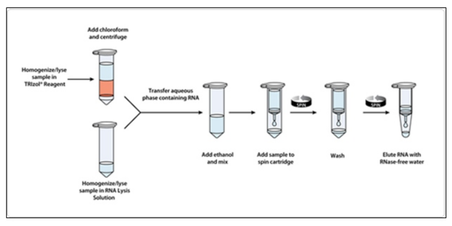
CELL LINES CULTURE

- **K562**
- **K562-R** (Kindly provided by Dra Flávia Vasconcelos and Dra Raquel Maia) *suplemented with 1µM IM
- **Lucena** (Kindly provided by Dra Vivian Rumjanek) *suplemented with 60nM Vincristine

Healthy bone marrow donor samples

RNA EXTRACTION

$1x10^6$ cells
Trizol (Invitrogen)
Rneasy Kit (Qiagen)



QUANTIFICATION OF RNA AND QUALITY CONTROL

1µL of RNA sample
Spectrophotometer ND-1000 (Nanodrop Technologies)
RIN (Nanochip - Agilent)

cDNA SYNTHESIS

2µg of RNA sample
DNAse treatment (Invitrogen)
SuperScript™ First-Strand Synthesis (Invitrogen)

Real Time Quantitative PCR

Intercalating dye Sybr (Thermo Fisher)
Thermocycler Rotor Gene (Qiagen)
Standard curves
Normalizing genes: *ACTB* and *B2M*

RESULTS AND PERSPECTIVES

HOTAIR is overexpressed in IM resistance in Chronic myelogenous leukemia

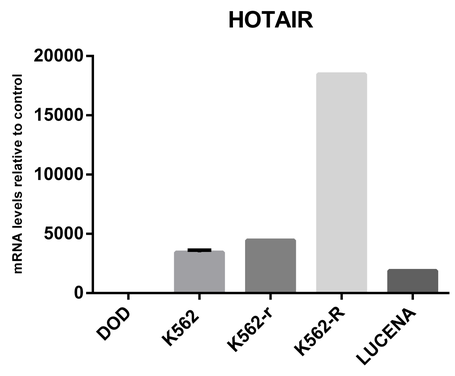
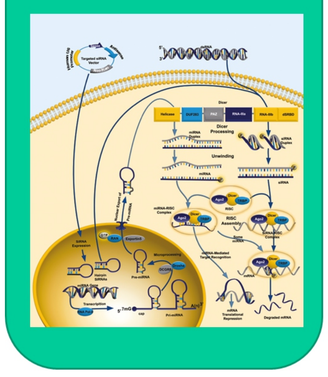


Figure 7. RT-qPCR analysis of mRNA levels of expression of the *HOTAIR* in DOD, healthy bone marrow donor samples, K562, responsive to IM treatment; K562-r, K562 in process of resistance aquisition; K562-R, resistant to IM (1M) and Lucena (Vincristine/cross-resistance to IM) cell lines. Total RNA was isolated and used in quantitative real-time PCR to determine changes *HOTAIR* expression levels after normalization to media of *ACTB* and *B2M* expression.

SILENCING OF HOTAIR

Small interfering RNA

Specific silence in K562-R cell line

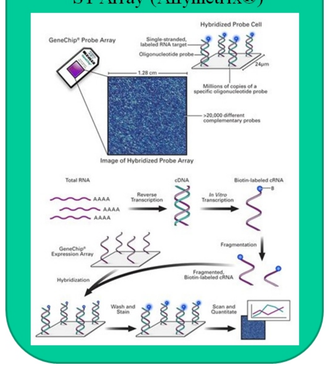


EVALUATION OF GENE EXPRESSION PROFILE

mRNA Chiparray

Comparing silenced K562-R *versus* K562-R (scrambled) and subsequently with K562-S

GeneChip ™ Human Gene 1.0 ST Array (Affymetrix®)



***IN SILICO* ANALYSIS**

- Direct and indirect analysis
- Networks
- Canonical ways
- Cellular functions
- Regulators

Validation of the obtained results will be carried out with specific assays

APOIO FINANCEIRO: CNPq, FAPERJ, Ministério da Saúde.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

MINISTÉRIO DA SAÚDE