PROTEOMIC ANALYSIS OF THE ASXL2 PROTEIN PARTNERS AND EVALUATION OF HISTONE METHYLTRANSFERASE ACTIVITY IN THE DIFFERENT BREAST CANCER SUBTYPES

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INTRODUCTION

Breast cancer (BC) is one of the largest public health issues in Brazil and worldwide, and its etiology is related to environmental, genetic and epigenetic factors, the latter being capable of promoting major alterations in gene expression. The antagonic protein families Polycomb (Pc) and Trithorax (Trx) are histone modifiers with an important role in controlling chromatin by promoting e.g. acetylation and methylation in specific amino acid residues. Two of the most known modifications are the methylations in H3K27 (Pc - silencing) and H3K4 (Trx - activation). Another family of proteins, ASXL, has been described as a potential regulator and/or enhancer of Pc and Trx activity. It has been suggested that these proteins can act in activation, repression, transcriptional regulation and recruitment of Pc and Trx members to their targets in chromatin. Still, histone modification is the least studied epigenetic mechanism, and little is known about the actions of Pc, Trx and ASXL in BC. BC can be stratified into molecular subtypes, varying in aggressiveness and prognosis, according to the expression of membrane receptors for estrogen, progesterone and HER2. This gives rise, respectively, to the Luminal A, Luminal B, HER2 and Triple Negative (TN) subtypes. In a previous study, we found the ASXL2 gene to be overexpressed in particular subtypes of BC cell lines, which indicates it might have a role in this disease.

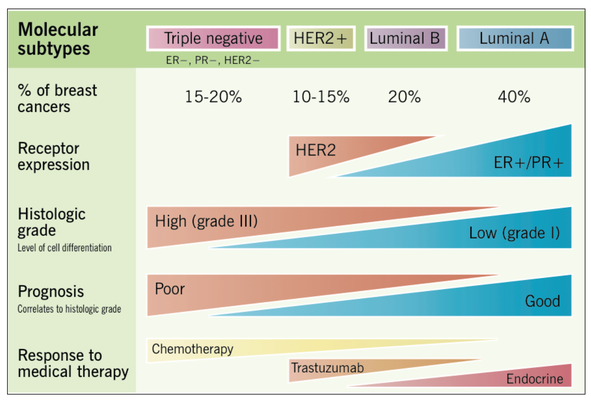


Figure 1: Classification of Breast Cancer molecular subtypes. Adapted from http://www.pathophys.org/breast-cancer/.

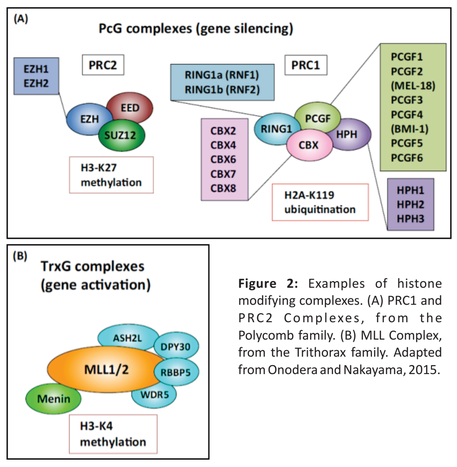


Figure 2: Examples of histone modifying complexes. (A) PRC1 and PRC2 Complexes, from the Polycomb family. (B) MLL Complex, from the Trithorax family. Adapted from Onodera and Nakayama, 2015.

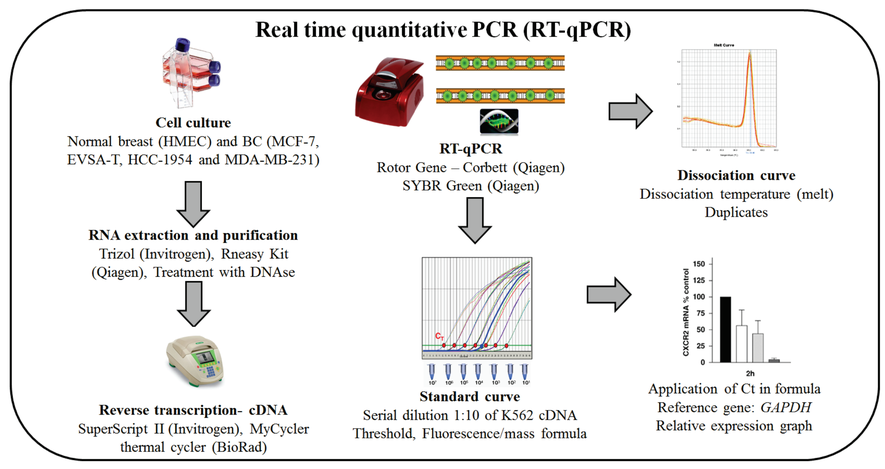
OBJECTIVE

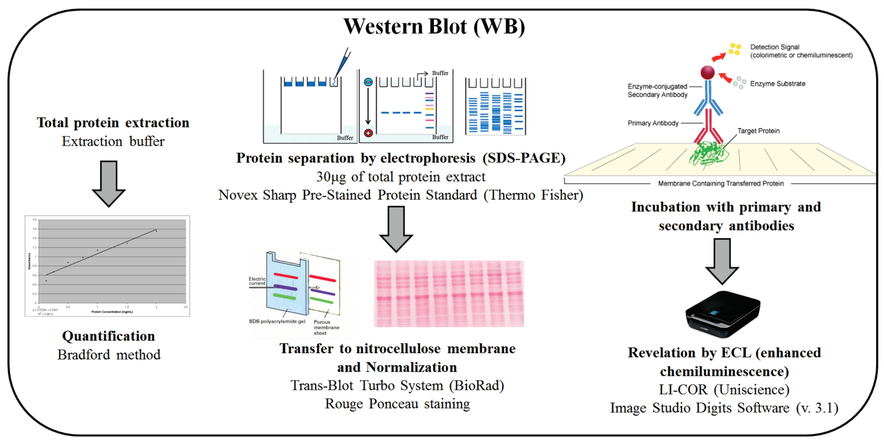
The aim of this study is to identify the potential role of ASXL2 in the epigenetic regulation of the BC subtypes.

METHODOLOGY

Table 1: Genes assessed in this study.

Polycomb Group		Trithorax Group			Other
PRC1 Complex	PRC2 Complex	MLL Complex	NURF Complex	SWI/SNF Complex	
BMI1	*EED*	*ASH2L*	*RBBP4*	*SMARCA2*	*ASXL1*
PHC3	*EZH1*	*KMT2A*	*SMARCA1*	*SMARCA4*	*ASXL2*
RING1	*EZH2*	*PRMT6*			
YY1	*SIRT1*	*WDR5*			





RESULTS

ASXL2 *in silico* analysis and investigation in BC cell lines

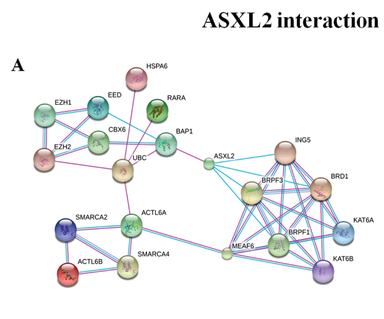


Figure 3: (A) *In silico* analysis of the ASXL2 protein interactions. Interaction map obtained from online database STRING v.10.0 (www.string-db.org) with following parameters: high confidence (0.900) and in silico/experimental data. (B) ASXL interactions with members from the Pc and Trx families in transcriptional repression and activation, respectively. Adapted from Katoh, 2013.

ASXL2 expression in BC cell lines

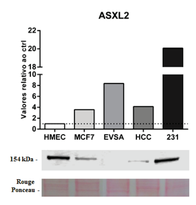
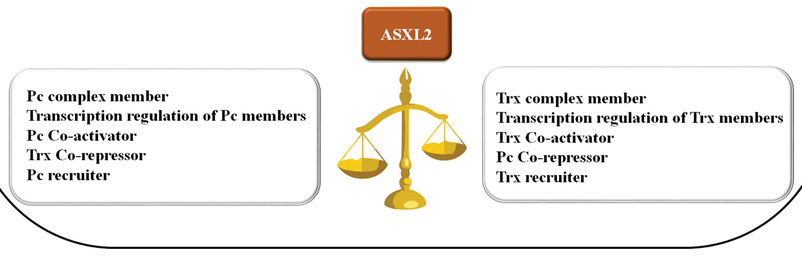


Figure 4: RT-qPCR and WB analysis of ASXL2 in BC cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.



Expression analysis of Pc and Trx families in BC

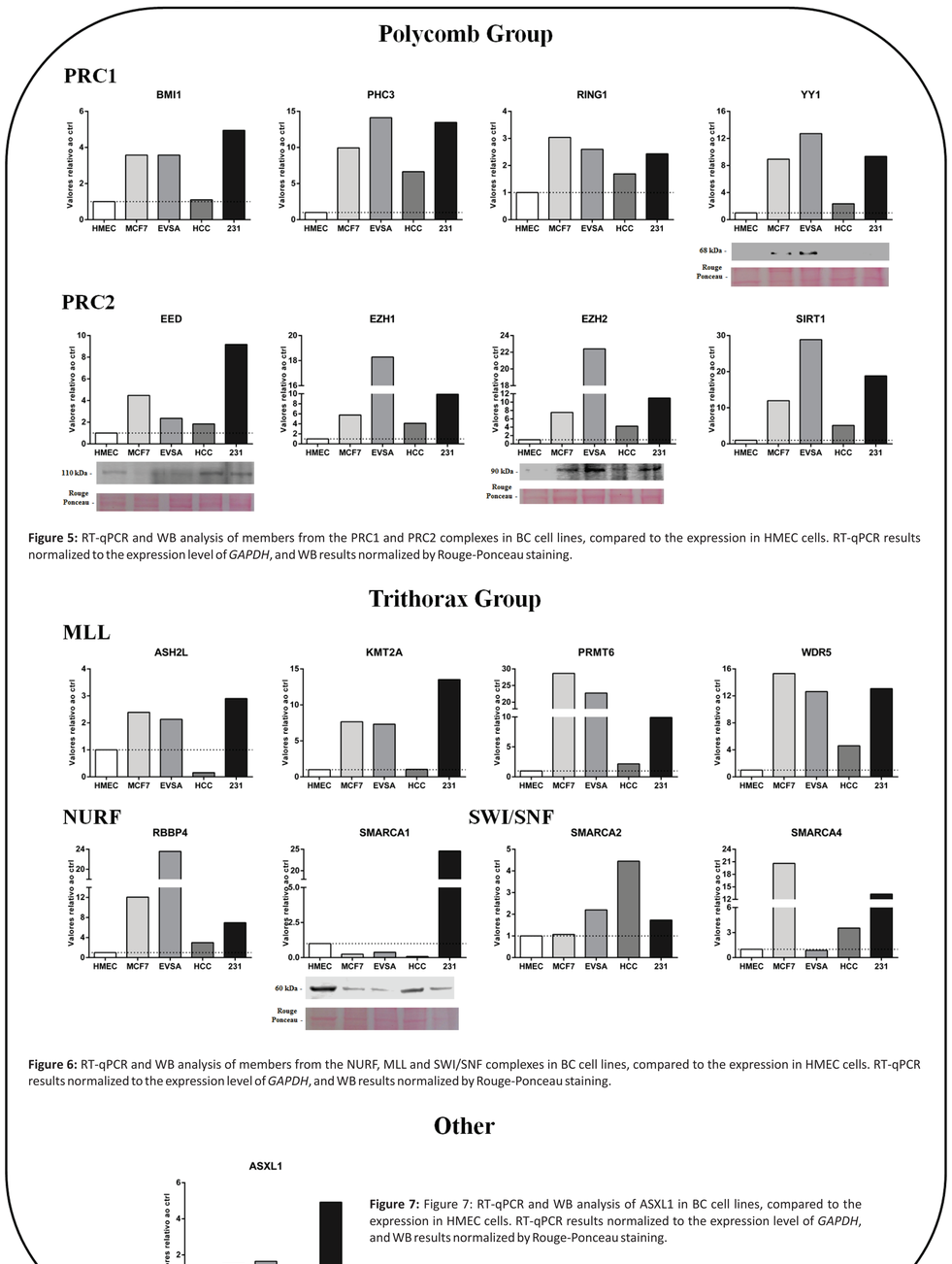


Figure 5: RT-qPCR and WB analysis of members from the PRC1 and PRC2 complexes in BC cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.

Figure 6: RT-qPCR and WB analysis of members from the NURF, MLL and SWI/SNF complexes in BC cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.

Figure 7: Figure 7: RT-qPCR and WB analysis of ASXL1 in BC cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.

PERSPECTIVES

To further understand the role of ASXL2 in the epigenetic regulation of BC, we will perform an immunoprecipitation assay followed by mass spectrometry, in order to identify ASXL2's partners in the BC cell lines. Also, we will carry out a methyltransferase activity assay in H3K27 (Pc) and H3K4 (Trx), aiming to correlate such activity to the action of the Pc and Trx proteins in BC.

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