IDENTIFICATION OF NOVEL PARTNERS OF ASXL2 PROTEIN BY HIGH-THROUGHPUT PROTEOMICS AND THEIR IMPLICATION IN CHROMATIN REMODELING IN BREAST CANCER SUBTYPES

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INTRODUCTION

Breast cancer's (BC) etiology can be related to epigenetic factors. Among them, chromatin remodeling is one of the least explored, and includes the action of the antagonic protein families Polycomb (Pc) and Trithorax (Trx), respectively involved in gene silencing and activation. Asx has been described as an enhancer of Pc and Trx activity in Drosophila development, with homologues of this protein described in humans (ASXL). ASXL2 has been reported in estrogen receptor-positive (ER+) BC, mediating ER activation and the transcription of its target genes by recruiting histone modifiers to the chromatin. In a previous study from our group, ASXL2 was found overexpressed in Luminal A (LUM A, ER+) and Triple Negative (TN, ER-) BC cell lines, respectively the least and most aggressive ones.

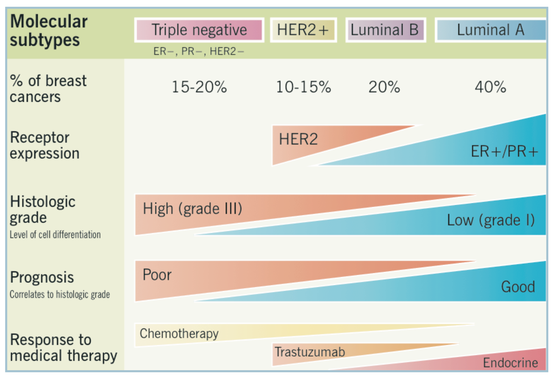


Figure 1: Classification of Breast Cancer molecular subtypes. Adapted from Wong and Rebelo, 2012.

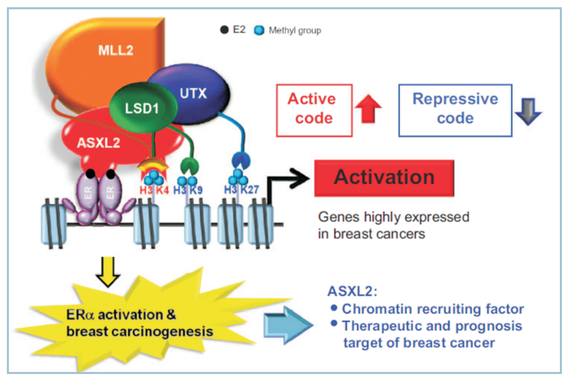


Figure 2: Model for the epigenetic role of ASXL2 in ER+ Breast Cancer. Adapted from Park *et al.*, 2016.

ASXL2 expression in BC cell lines

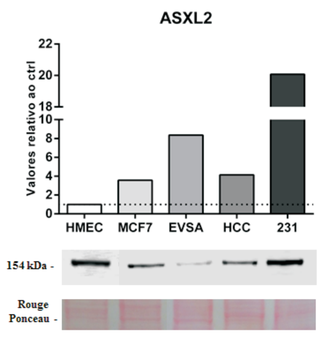


Figure 3: RT-qPCR and WB analysis of ASXL2 in BC cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.

ASXL2 interacts with Pc and Trx members in Development

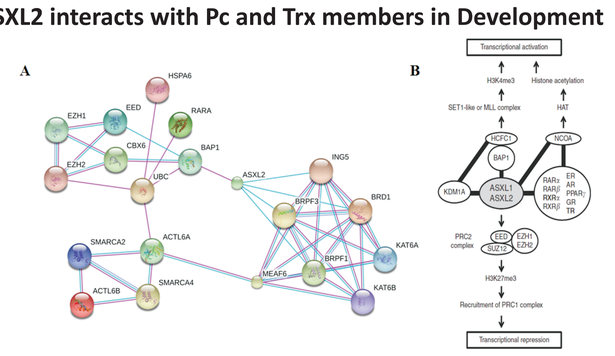


Figure 4: ASXL2 interacts with Pc and Trx members in Development. (A) *In silico* analysis of the ASXL2 protein interactions. Interaction map obtained from online database STRING v.10.0 (www.string-db.org) with following parameters: high confidence (0.900) and *in silico*/experimental data. (B) ASXL interactions with members from the Pc and Trx families in transcriptional repression and activation, respectively. Adapted from Katoh, 2013.

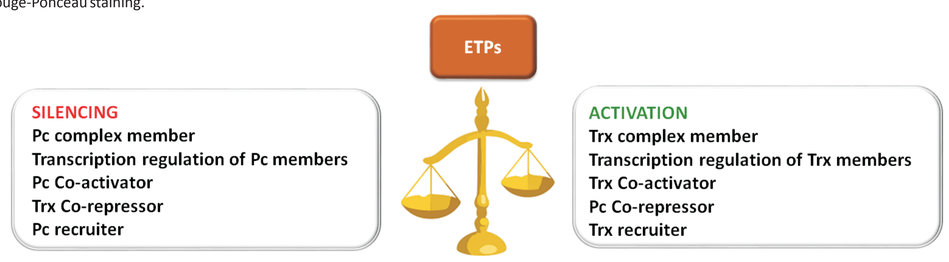


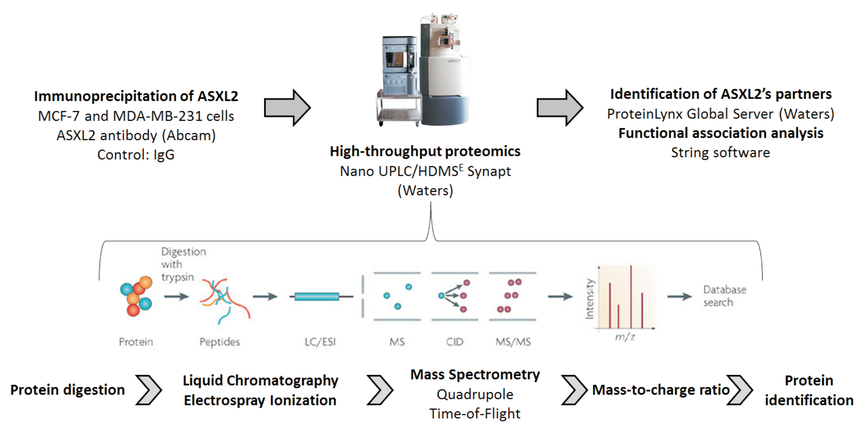
Figure 5: Model for the dual role of Enhancer of Trithorax and Polycomb (ETP) proteins, including the ASXL2 protein, in promoting/facilitating both gene silencing and activation.

OBJECTIVE

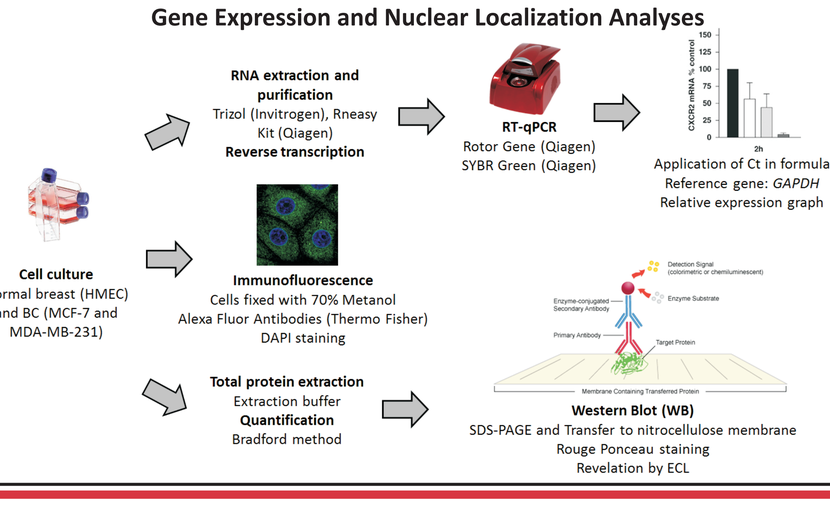
The aim of this study is to identify the partners and potential role of ASXL2 in the epigenetic regulation of BC.

METHODOLOGY

Protein Immunoprecipitation and Proteomic Analysis



Gene Expression and Nuclear Localization Analyses



RESULTS

Proteomic analysis evidences ASXL2 has different partners in LUM A and TN cells

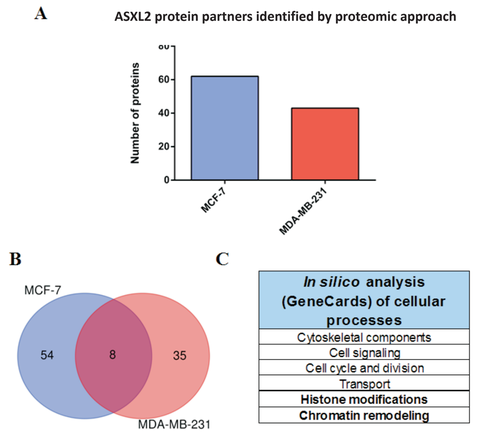


Figure 6: ASXL2 is related with histone modifiers in BC. (A) Protein identification of ASXL2's partners through proteomic analysis in MCF-7 (LUM A) and MDA-MB-231 (TN) cells after ASXL2 imumnoprecipitation (n=3). Results normalized to IgG control. (B) Venn diagram of the proteins immunoprecipitated with ASXL2 in MCF-7 and MDA-MB-231 cells. (C) List of cellular processes in which the identified proteins are involved, obtained via *in silico* analysis through the GeneCards website (www.genecards.org).

Table 1: List of proteins immunoprecipitated with ASXL2 in MCF-7 cells.

MCF-7	
Protein symbol	**Protein name**
ACTBL2	Beta-actin-like protein 2
ANK2	Ankyrin-2
ANK3	Ankyrin-3
CCDC125	Coiled-coil domain-containing protein 125
CFL1	Cofilin-1
CNTRL	Centriolin
COPS7B	COP9 signalosome complex subunit 7b
CUBN	Cubilin
DNHD1	Dynein heavy chain domain-containing protein 1
DOCK10	Dedicator of cytokinesis protein 10
DOCK7	Dedicator of cytokinesis protein 7
EEF1A2	Elongation factor 1-alpha 2
EEF2	Elongation factor 2
EHBP1	EH domain-binding protein 1
ENO1	Alpha-enolase
ENO2	Gamma-enolase
ENO3	Beta-enolase
FAM47E	Protein FAM47E
FASN	Fatty acid synthase
FLNA	Filamin-A
G6PD	Glucose-6-phosphate 1-dehydrogenase
GAK	Cyclin-G-associated kinase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
HSP90AB4P	Putative heat shock protein HSP 90-beta 4
HSP9B1	Endoplasmin
HSPA9	Stress-70 protein, mitochondrial
JADE1	Protein Jade-1
KDM8	JmjC domain-containing protein 5
KRT1	Keratin, type II cytoskeletal 1
KRT18	Keratin, type I cytoskeletal 18
KRT4	Keratin, type II cytoskeletal 4
KRT7	Keratin, type II cytoskeletal 7
KRT80	Keratin, type II cytoskeletal 80
MAP3K11	Mitogen-activated protein kinase kinase kinase 11
MYO5A	Unconventional myosin-Va
MYO9A	Unconventional myosin-IXa
NCL	Nucleolin
NPM1	Nucleophosmin
PCBP2	Poly(rC)-binding protein 2
PKM	Pyruvate kinase PKM
POTEE	POTE ankyrin domain family member E
POTEF	POTE ankyrin domain family member F
POTEKP	Putative beta-actin-like protein 3
PPIA	Peptidyl-prolyl cis-trans isomerase A
PRDX1	Peroxiredoxin-1
RAPGEF4	Rap guanine nucleotide exchange factor 4
RPL6	60S ribosomal protein L6
RPLP1	60S acidic ribosomal protein P1
SLC3A2	4F2 cell-surface antigen heavy chain
SREK1	Splicing regulatory glutamine/lysine-rich protein 1
TF	Serotransferrin

Table 2: List of proteins immunoprecipitated with ASXL2 in MDA-MB-231 cells.

MDA-MB-231	
Protein symbol	**Protein name**
ANKRD36	Ankyrin repeat domain-containing protein 36A
BAZ1A	Bromodomain adjacent to zinc finger domain protein 1A
CCDC88C	Protein Daple
CCT2	T-complex protein 1 subunit beta
CFL1	Cofilin-1
DOCK8	Dedicator of cytokinesis protein 8
EEF2	Elongation factor 2
EIF5A	Eukaryotic translation initiation factor 5A-1
EPHA6	Ephrin type-A receptor 6
FLNC	Filamin-C
GFAP	Glial fibrillary acidic protein
IFI44	Interferon-induced protein 44
IL1R1	Interleukin-1 receptor type 1
KRT1	Keratin, type II cytoskeletal 1
KRT10	Keratin, type I cytoskeletal 10
KRT2	Keratin, type II cytoskeletal 2 epidermal
KRT4	Keratin, type II cytoskeletal 4
KRT5	Keratin, type II cytoskeletal 5
KRT6A	Keratin, type II cytoskeletal 6A
KRT6B	Keratin, type II cytoskeletal 6B
KRT6C	Keratin, type II cytoskeletal 6C
KRT7	Keratin, type II cytoskeletal 7
KRT77	Keratin, type II cytoskeletal 1b
KRT8	Keratin, type II cytoskeletal 8
KRT80	Keratin, type II cytoskeletal 80
LDHB	L-lactate dehydrogenase B chain
PPFIA1	Liprin-alpha-1
PRRC2C	Protein PRRC2C
PTBP1	Polypyrimidine tract-binding protein 1
RAPGEF2	Rap guanine nucleotide exchange factor 2
RPGRIP1L	Protein fantom
RSBN1L	Round spermatid basic protein 1-like protein
SFN	14-3-3 protein sigma
TBC1D9B	TBC1 domain family member 9B
TF	Serotransferrin
TRIM17	E3 ubiquitin-protein ligase TRIM17
VIM	Vimentin
YWHAB	14-3-3 protein beta/alpha
YWHAE	14-3-3 protein epsilon
YWHAG	14-3-3 protein gamma
YWHAH	14-3-3 protein eta
YWHAQ	14-3-3 protein theta
YWHAZ	14-3-3 protein zeta/delta

Interactome of ASXL2's partner proteins in MCF-7 cells

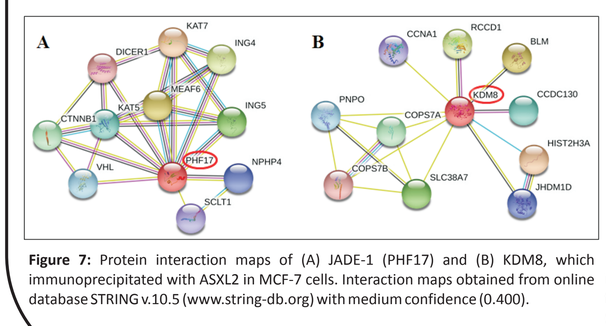


Figure 7: Protein interaction maps of (A) JADE-1 (PHF17) and (B) KDM8, which immunoprecipitated with ASXL2 in MCF-7 cells. Interaction maps obtained from online database STRING v.10.5 (www.string-db.org) with medium confidence (0.400).

Interactome of ASXL2's partner proteins in MDA-MB-231 cells

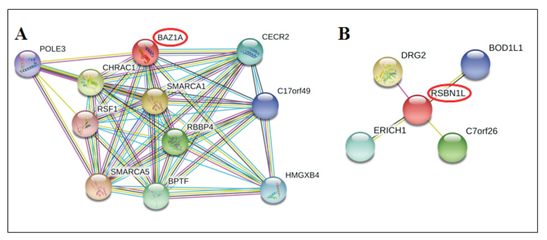


Figure 8: Protein interaction maps of (A) BAZ1A and (B) RSBN1L, which immunoprecipitated with ASXL2 in MDA-MB-231 cells. Interaction maps obtained from online database STRING v.10.5 (www.string-db.org) with medium confidence (0.400).

Pc and Trx members express and localize differently in LUM A and TN cells

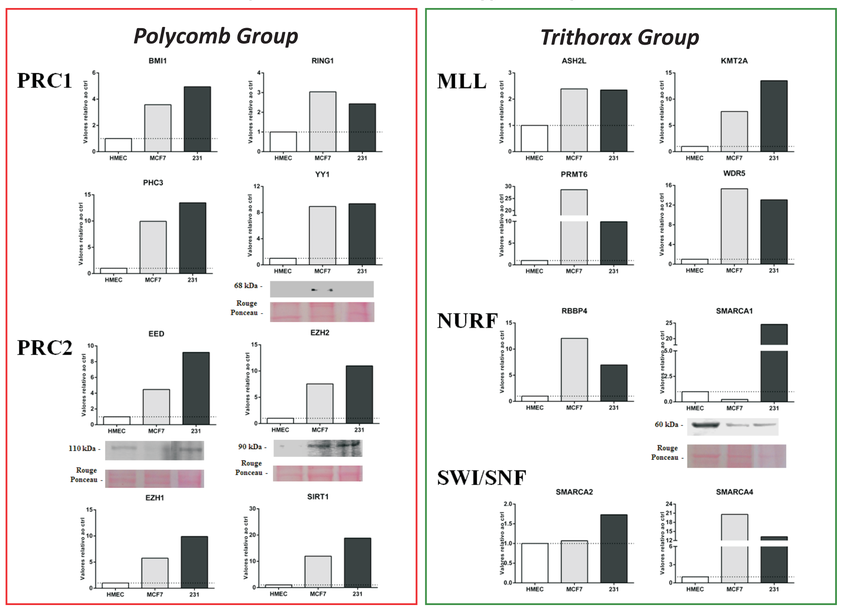


Figure 9: RT-qPCR and WB analysis of members from the Polycomb and Trithorax complexes in LUM A and TN cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining.

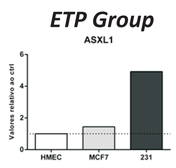


Figure 10: RT-qPCR and WB analysis of ASXL1 in LUM A and TN cell lines, compared to the expression in HMEC cells. RT-qPCR results normalized to the expression level of *GAPDH*, and WB results normalized by Rouge-Ponceau staining

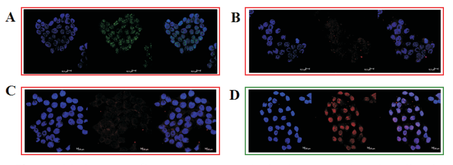


Figure 11: Immunofluorescence analysis shows nuclear localization of Pc group proteins (A) YY1 (green), (B) EED (red), (C) EZH2 (red) and Trx group protein (D) SMARCA1 (red) in MCF-7 cells (63x). Cells fixed with 70% Metanol and nuclei stained with DAPI (blue). Results were obtained using confocal laser scanning microscopy.

CONCLUSION

Overall, our results pointed to chromatin modifiers and remodelers poorly investigated in BC as potential partners of ASXL2 and suggest differential action of Pc and Trx members in LUM A and TN cells. Validation of obtained results, as well as functional assays, will be carried out in order to provide further comprehension on ASXL2's epigenetic role in BC.

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

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