STUDY OF CALPAIN 10 ROLE IN BREAST CANCER AGGRESSIVENESS

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INTRODUCTION

Breast cancer (BC) is the most common among women worldwide. It is a complex disease and exhibits a wide scope of molecular, histological and clinical features. Classification is based on the expression of estrogen receptor (ER), progesterone (PR) and human epidermal factor 2 receptor (HER2). Therefore, BC is stratified into Luminal A, Luminal-B, HER2 positive (HER2+) and Triple-negative (TN) molecular subtypes, being HER2 and TN more aggressive and with worse prognosis. Despite the improvement in clinics, this classification is not yet capable of encompassing all molecular alterations, since there is a great heterogeneity intra/extra-subtypes, requiring more studies to understand BC. The calpain family consists of 15 members (typical and atypical) of cysteine proteinases capable of several substrates cleaveage. The named typical calpains (CAN1 and CAN2) have been extensively studied and related with tumorigenic processes and correlated with tumor aggressiveness. In BC it has already been reported that CAN1 and CAN2 are able to cleave the HER2 receptor in in vitro models, altering sensitivity to treatment with Trastuzumab. A proteomic study performed in our laboratory reported high levels of calpain 10 (CAN10) in the blood plasma of patients classified as HER2- tumors, and this finding was confirmed in biopsies of these patients. It has also been shown that the in vitro models of HER2+ (HCC-1954) and TN (MDA-MB-231) have increased CAN10 expression. Interestingly, MDA-MB-231 cells present the intracellular portion of the HER2 receptor phosphorylated, indicating a possible HER2 signaling activity; moreover the non-specific blocking of CAN10, caused an overexpression of this phosphorylation level. However, CAN10 function and activity is still uncovered in BC.

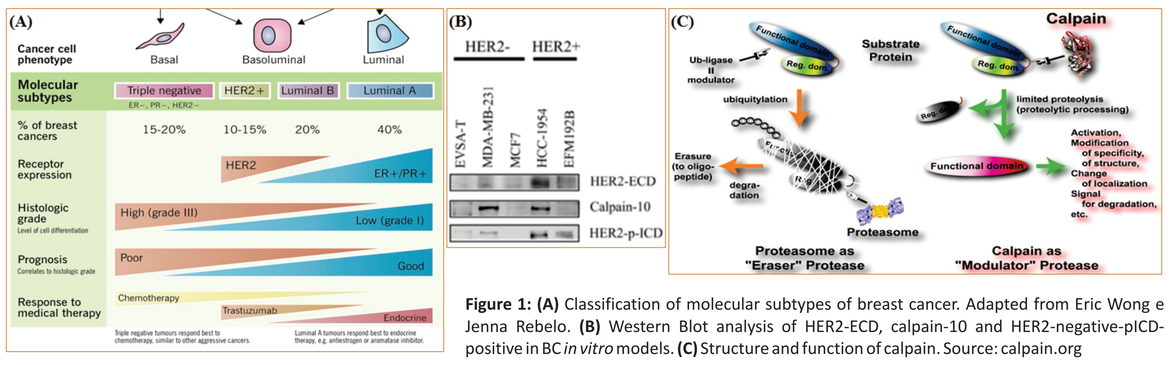
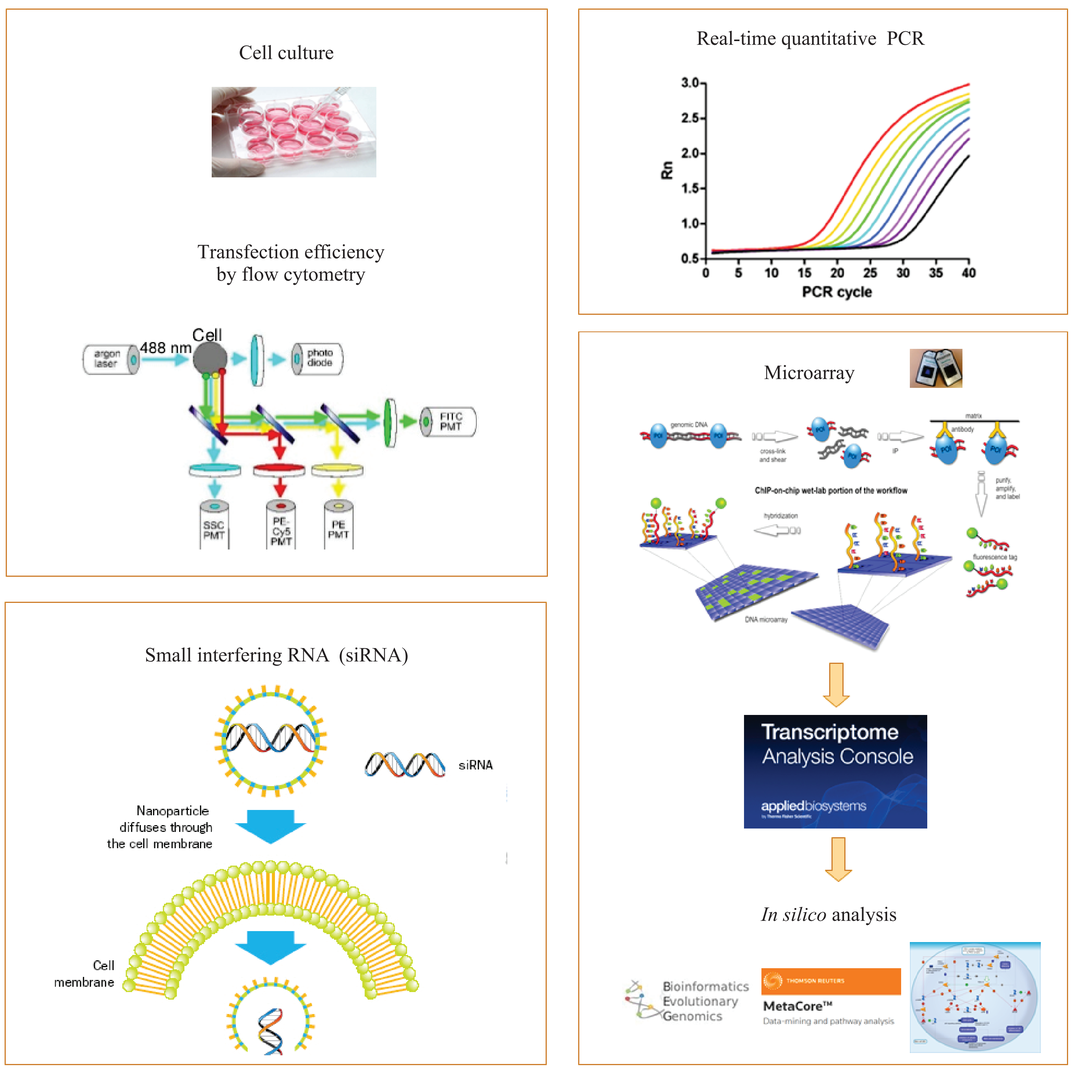


Figure 1: (A) Classification of molecular subtypes of breast cancer. Adapted from Eric Wong e Jenna Rebelo. **(B)** Western Blot analysis of HER2-ECD, calpain-10 and HER2-negative-pICD-positive in BC *in vitro* models. **(C)** Structure and function of calpain. Source: calpain.org

OBJECTIVE

The present study aims to identify the signaling pathways in which calpain 10 can act in BC, and correlate them with the aggressiveness of the disease.

METHODOLOGY



FINANCIAL SUPPORT: Ministério da Saúde – INCA, FAPERJ e CNPq.

RESULTS

Transient Silencing of CAPN10 in HER2+ and TN BC models

1) Transfection efficiency with lipofectamine:

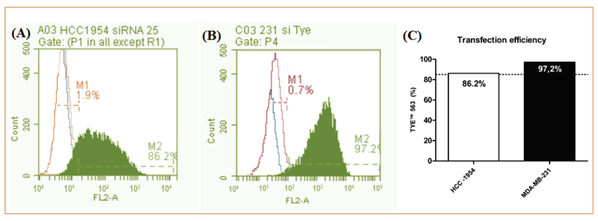


Figure 1: Analysis of transfection efficiency by flow cytometry. Representative histograms from **(A)** HCC-1954 cells **(B)** MDA-MB-231 cells transfected with TYE-563 labeled siRNA. **(C)** Transfection efficiency using 2.5 µg lipofectamine and 25 nM TYE-563 labeled. Both cell lines presented more than 95% cellular viability. Control (blue), mock (red) and TYE-563 labelled siRNA (green).

2) Transient silencing of CAPN10:

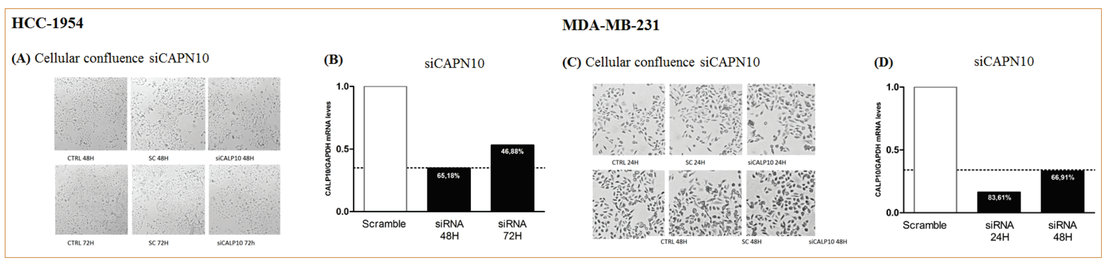


Figure 2: : siRNA CAPN10 efficiency. (A) HCC-1954 cells confluence after transfection (48 and 72 h) and **(C)** MDA-MB-231 cells confluence after transfection (24 and 48 h), using 2.5 µg lipofectamine and 50 nM siCAPN10. Analysis of *CAPN10* mRNA levels after after siRNA in **(B)** HCC-1954 cells and **(D)** MDA-MB-231 cells. Total RNA was isolated and used in RT-qPCR analysis to determine changes in *CAPN10* mRNA levels after nomarlization with GAPDH expression. CTRL: control. SC: scramble. siCAPN10: siRNA CAPN10

Potential genes, pathways and signaling related to Calpain 10 in HER2+ and TN BC models

1) Differentially expressed genes in siCAPN10:

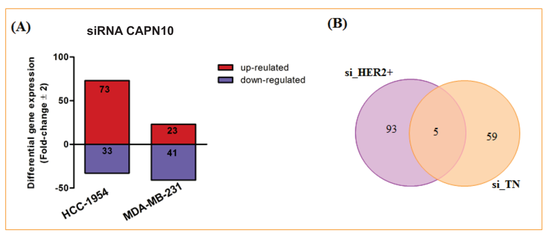


Figure 3: Differential gene expression in siCAPN10. (A) Differentially expressed genes (up and down-regulated) after siCAPN10 in HCC-1954 and MDA-MB-231 cells with cut-off +/-2. **(B)** Venn diagram showing the comparative analysis of the si_HER2+ (HCC-1954) and si_TN (MDA-MB-231).

Table 1: Top 10 canonical pathway maps from siCAPN10 in BC models:

	siRNA CAPN10 HCC-1954	siRNA CAPN10 MDA-MB-231
1	Inter-cellular relations in COPD	EthanolAcetaldehyde-dependent stimulation of MMP-9 expression in HCC
2	Cytoskeleton remodeling - Hyaluronic acid CD44 signaling pathways	Regulation of VEGF signaling in pancreatic cancer
3	Immune response - Histamine H1 receptor signaling in immune response	Regulation of angiogenesis in prostate cancer
4	PDE4 regulation of cytochemokine expression in arthritis	Neurophysiological process - Synaptic vesicle fusion and recycling in nerve terminals
5	Chemotaxis - Inhibitory action of lipoxins on IL-8- and Leukotriene B4-induced neutrophil migration	Alcohol metabolism predisposition of HCC development
6	Cell adhesion - ECM remodeling	Role of epigenetic alterations in proliferation and differentiation of SCLC cells
7	Immune response - IL-33 signaling pathway	Cell adhesion - Integrin inside-out signaling in neutrophils
8	Immune response - TREM1 signaling pathway	Immune response - Sialic-acid receptors (Siglecs) signaling
9	Immune response - CD16 signaling in NK cells	Involvement of VEGF signaling in the progression of lung cancer
10	Transport - Clathrin-coated vesicle cycle	DeltaF508-CFTR traffic ER-to-Golgi in CF

2) In silico analysis of pathways and signaling related to Calpain 10:

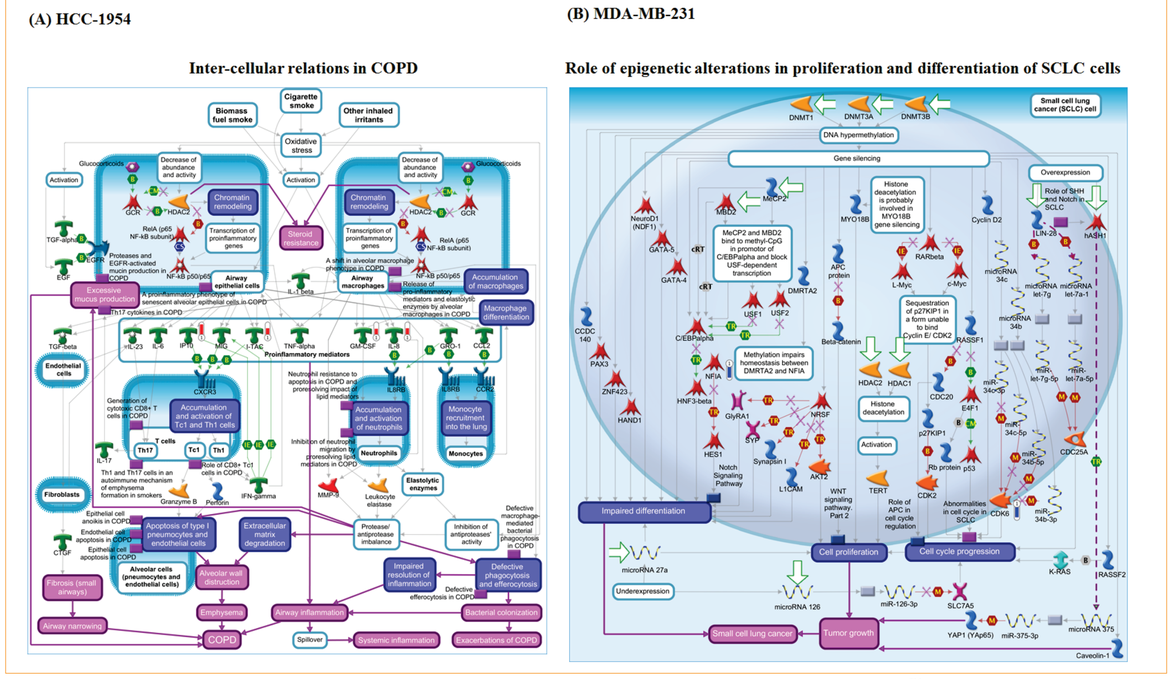


Figure 4: Representative pathway maps from siCAPN10 in HCC-1954 and MDA-MB-231 cells. *In silico* analysis using MetaCore™ *software* (GeneGO Inc., Encinitas, CA) exposed different pathways from siCAPN10. **(A)** Inter-cellular relations in COPD pathway **(B)** Role of epigenetic alterations in proliferation and differentiation of SCLC cells pathways.

CONCLUSION AND PERSPECTIVES

Through the data obtained so far, CAN10 plays different roles in HER2 and TN subtypes. Confirmation of the exposed pathways and signaling through biological assays may add information of direct or indirect activity related to Calpain 10 in BC.

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