STUDY OF CALPAIN 10 ROLE IN BREAST CANCER AGGRESSIVENESS

Andressa Marques(ME), Renata Binato (PhD), Eliana Abdelhay (PhD), Stephany Corrêa (PhD)
Laboratório de Célula Tronco, Instituto Nacional de Câncer (INCA)
Email: aferrazpm@hotmail.com

INTRODUCTION

Breast cancer (BC) molecular subtypes HER2+ and Triple-Negative (TN) are the most aggressive with worse prognosis. Despite the improvement in clinics, there is a great heterogeneity intra/extra-subtypes, requiring molecular studies to understand BC. A previous proteomic study from our group reported high levels of calpain 10 (CAN10) in patients'plasma (HER2-), compared to HER2+. 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 blockage of CAN10, lead to an overexpression of such level. However, CAN10 function and activity is still uncovered in BC.

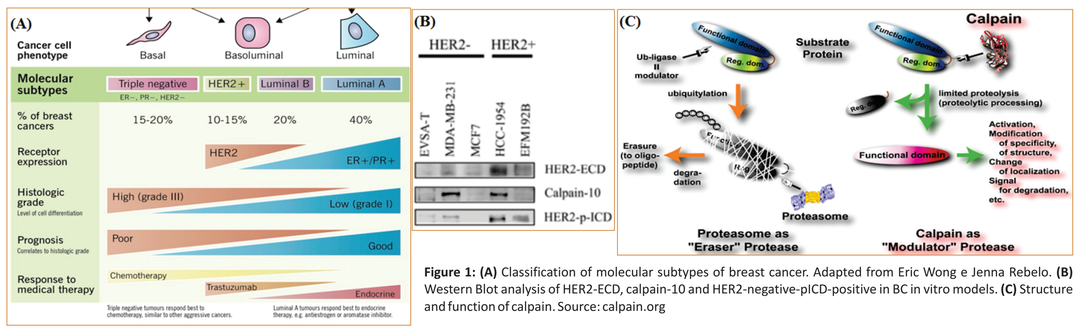
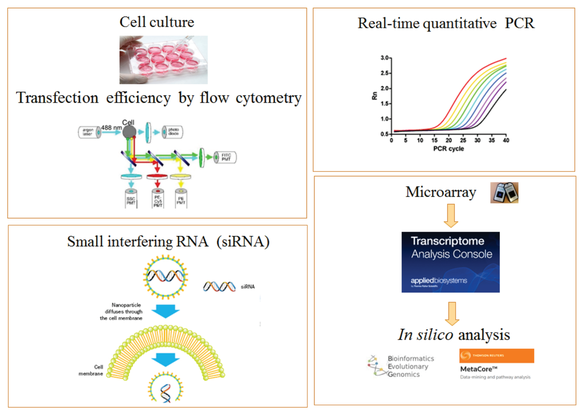


Figure 1: (A) Classification of molecular subtypes of breast cancer. Adapted from Eric Wong e Jenna Rebelo. **(B)** Western Blot analysis of HER2-ECD, calpain-10 and HER2-negative-pICD-positive in BC in vitro models. **(C)** Structure and function of calpain. Source: calpain.org

METHODOLOGY



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.

RESULTS

Transient Silencing of CAPN10 in HER2⁺ and TN BC models

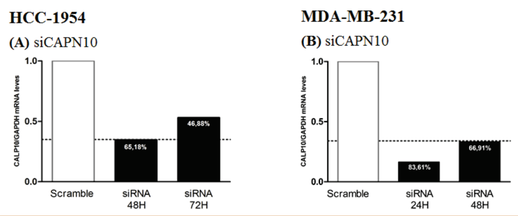


Figure 2: siRNA CAPN10 efficiency. (A) HCC-1954 cells and **(B)** MDA-MB-231 cells. Total RNA was isolated and used in RT-qPCR analysis to determine changes in *CAPN10* mRNA levels after nomarlization with *GAPDH* expression. CTRL: control. SC: scramble. siCAPN10: siRNA CAPN10

The intrisic role of Calpain 10 in HER2⁺ BC model

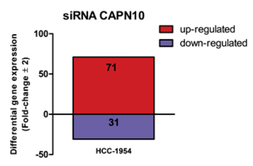


Figure 3: Differential gene expression in siCAPN10 HCC-1954. Differentially expressed genes (up and down-regulated) after si*CAPN10* in HCC-1954 cells with cut-off +/-2.

Table 1: Top 10 canonical pathway maps from siCAPN10 HER2+ BC models:

	siRNA CAPN10 HCC-1954
1	Inter-cellular relations in COPD
2	Cytoskeleton remodeling - Hyaluronic acid CD44 signaling pathways
3	Immune response - Histamine H1 receptor signaling in immune response
4	PDE4 regulation of cytochemokine expression in arthritis
5	Chemotaxis - Inhibitory action of lipoxins on IL-8- and Leukotriene B4-induced neutrophil migration
6	Cell adhesion - ECM remodeling
7	Immune response - IL-33 signaling pathway
8	Immune response - TREM1 signaling pathway
9	Immune response - CD16 signaling in NK cells
10	Transport - Clathrin-coated vesicle cycle

The intrisic role of Calpain 10 in TN BC model

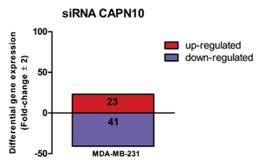


Figure 4: Differential gene expression in siCAPN10 MDA-MB-231. Differentially expressed genes (up and down-regulated) after si*CAPN10* in MDA-MB-231 cells with cut-off +/-2.

Table 2: Top 10 canonical pathway maps from si*CAPN10* TN BC models:

	siRNA CAPN10 MDA-MB-231
1	EthanolAcetaldehyde-dependent stimulation of MMP-9 expression in HCC
2	Regulation of VEGF signaling in pancreatic cancer
3	Regulation of angiogenesis in prostate cancer
4	Neurophysiological process - Synaptic vesicle fusion and recycling in nerve terminals
5	Alcohol metabolism predisposition of HCC development
6	Role of epigenetic alterations in proliferation and differentiation of SCLC cells
7	Cell adhesion - Integrin inside-out signaling in neutrophils
8	Immune response - Sialic-acid receptors (Siglecs) signaling
9	Involvement of VEGF signaling in the progression of lung cancer
10	DeltaF508-CFTR traffic ER-to-Golgi in CF

Calpain 10 is enrolled in different pathways in HER2+ and TN BC models

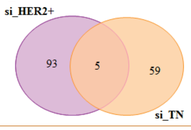
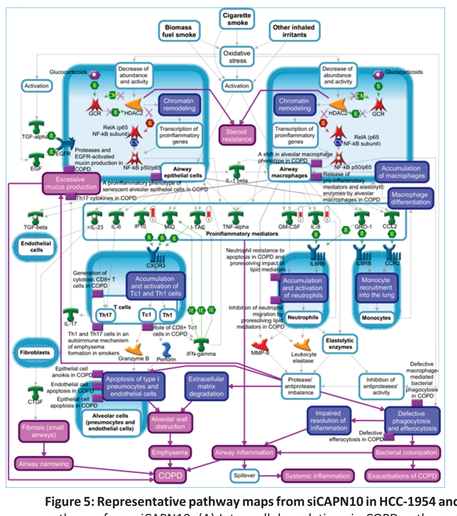


Figure 5: Differential gene expression in siCAPN10. Venn diagram showing the comparative analysis of the si_HER2+ (HCC-1954) and si_TN (MDA-MB-231).

In silico analysis of signaling pathways related to Calpain 10

(A) HCC-1954: Inter-cellular relations in COPD



(B) MDA-MB-231: Role of epigenetic alterations in proliferation and differentiation of SCLC cells

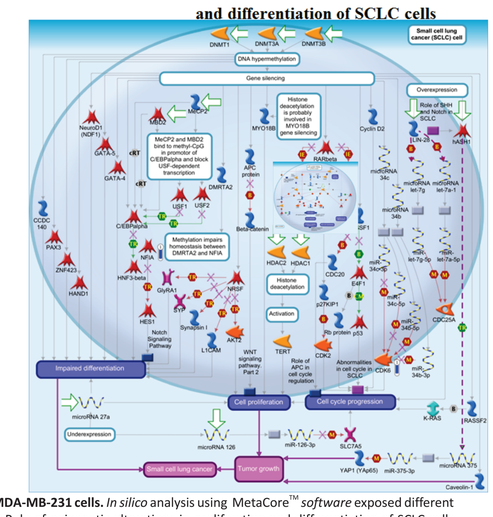


Figure 5: Representative pathway maps from siCAPN10 in HCC-1954 and MDA-MB-231 cells. *In silico* analysis using MetaCore™ *software* exposed different pathways from siCAPN10. (A) Inter-cellular relations in COPD pathway (B) Role of epigenetic alterations in proliferation and differentiation of SCLC cells pathways.

Genes altered by CAN10 among HER2⁺ and TN models

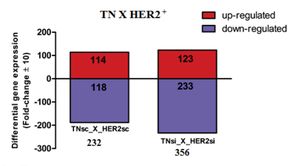


Figure 6: Differential gene expression in scramble and siCAPN10 HER2+ and TN models. Differentially expressed genes (up and down-regulated) of non silenced models and after si*CAPN10* in HER2+ and TN models with cut-off +/-10.

Figure 7: Genes alterad by CAN10 among HER2+ and TN models. Venn diagram showing the comparative analysis of the scTN X scHER2 + and siTN X siHER2+.

CAN10 may be related with EMT in TN BC model

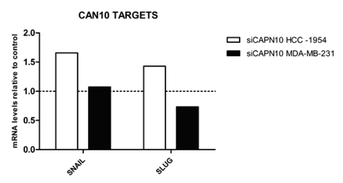


Figure 8: Canonical EMT markers as possib*le CAN10 targets in BC models.* *mRNA levels of SNAIL and* SLUG after nomarlization with **GAPDH** expression in si*CAPN10* HCC-1954 and si*CAPN10* MDA-MB-231.

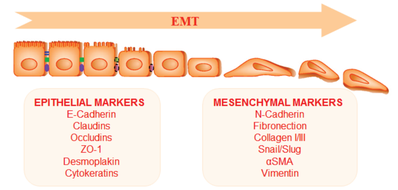


Figure 9: Canonical epithelial and mesenchymal markers. Adapted from Angadi Punnya and Kale Alka, 2015.

CAN10 influence in apoptosis and proliferation mediators in BC models

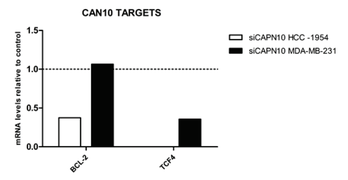


Figure 10: Canonical apoptosis and proliferation markers as possible CAN10 targets in BC models. mRNA levels of *BCL-2* and *TCF4* after nomarlization with *GAPDH* expression in si*CAPN10* HCC-1954 and si*CAPN10* MDA-MB-231.

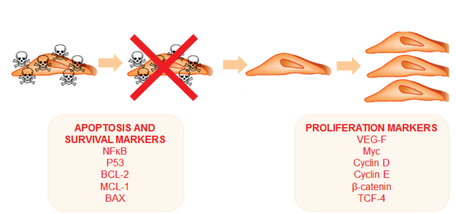


Figure 11: Canonical apoptosis, survival and proliferation markers. Adapted from Angadi Punnya and Kale Alka, 2015.

Typical calpains as possible CAN10 targets

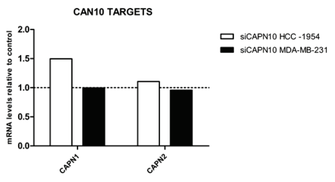


Figure 12: Typical calpains as possible CAN10 targets in BC models. mRNA levels of *CAPN1* and *CAPN2* after nomarlization with *GAPDH* expression in si*CAPN10* HCC-1954 and si*CAPN10* MDA-MB-231.

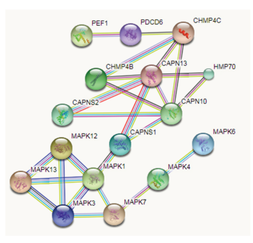


Figure 13: Network interacting proteins for CAN10. STRING network interaction showing 6 canonical proteins that interact directly with CAN10, including the small subunit of calpain 1 and calpain 2. From http://version10.5.string-db.org.

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.

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

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