STUDY OF CALPAIN 10 INVOLVEMENT IN BC AGGRESSIVENESS BY GENETIC AND EPIGENETIC MECHANISMS

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INTRODUCTION

Breast cancer (BC) classification is based on molecular subtypes with extrinsic/intrinsic heterogeneity. A previous work by our group shown that cellular models of HER2+ (HCC-1954) and Triple negative -TN (MDA-MB-231) subtypes, overexpress Calpain 10 (CAN10). Calpains are endoproteases capable to promote activation/inactivation of several substrats, eg. cytosolic enzimes, structural proteins and transcription factors (TFs). However, little is known about the function of CAN10 in BC. In this context, we previously perfomed a specific silencing (siCAN10) in HER2+ and TN models. After comparison of gene expression profile with controls, we identified differentially expressed (DE) genes related to tumorigenesis. *In silico* analysis by Metacore™ software showed that these transcripts may be regulated by TFs such as NFκB, cJun, cFos, cMyc and p53 described as targets of Calpains 1 and 2 (CAN1/2). Moreover, we also identified lncRNAs and miRNAs as DE, the latter being potentially regulated by NFκB and cMyc. As miRNAs and TFs may promote gene expression regulation in large-scale basis, it is important to dissect the direct targets of CAN10 and their relationship with observed DE genes. Moreover, it has been reported that CAN1/2 may activate/inactivate miRNAs biogenesis machinary, so it is ought to perform a focused investigation related to miRNAs expression.

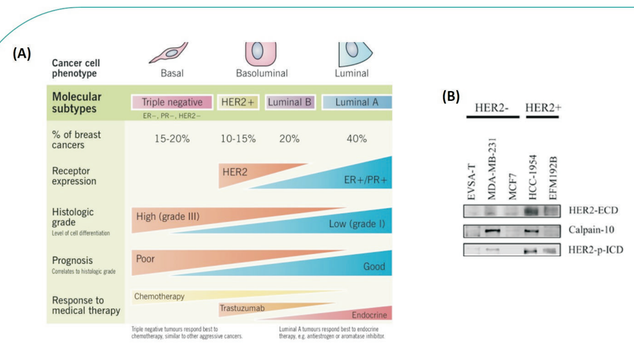


Figure 1: (A) Classification of molecular subtypes of breast cancer. Adapted from Eric Wong e Jenna Rebelo. **(B)** Western Blot analysis of HER2-ECD, calpain-10 and HER2-negative-pICD-positive in BC *in vitro* models. **(C)** Structure and function of calpain. Source: calpain.org

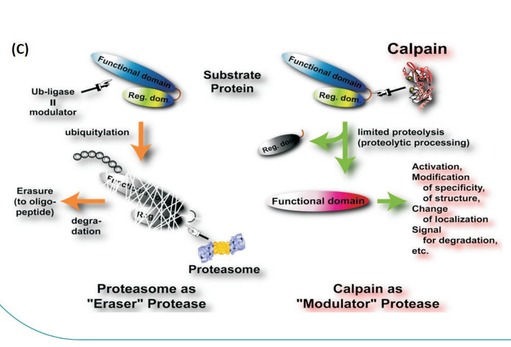


Table 1: Some of the target substrates of Calpain 1 and 2.

PROTEIN FUNCTIONS	CALPAIN TARGETS
Cytoskeletal/structural proteins	Actinin, Vimentin, Tubulin,
Integral membrane proteins	HER2, N-Cadherin, Progesterone Receptor
Enzimes	Protein Kinase C, Dicer, Drosha
Metabolic enzimes	Glycongen Synthase C, Piruvate Kinase
Transcription Factors	c-FOS, c-JUN, c-MYC, p53, NFκB

PREVIOUS RESULTS

CAN10 may influence gene expression in HER2+ and TN models

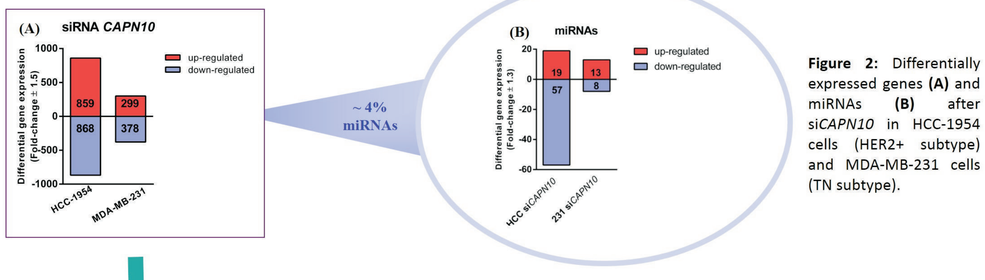


Figure 2: Differentially expressed genes **(A)** and miRNAs **(B)** after si*CAPN10* in HCC-1954 cells (HER2+ subtype) and MDA-MB-231 cells (TN subtype).

Table 2: Transcription factors that potentially regulate the DE genes

HCC-1954 *siCAPN10*	MDA-MB-231 *siCAPN10*
CREB1	CREB1
c-Myc	c-Myc
SP1	p53
ESR1	SP1
p53	ESR1
YY1	Oct-3/4
NF-kB	AP1 (c-JUN)
Oct-3/4	Androgen receptor
AP1 (c-JUN)	NF-kB
Androgen receptor	HIF1A

Table 3: Transcription factors that potentially regulate the DE miRNAs

HCC-1954 *siCAPN10*	MDA-MB-231 *siCAPN10*
c-Myc	p53
p53	HIF1A
STAT3	TCF8
RelA (p65 NF-kBsubunit)	c-Myc
E2F1	SIP1 (ZFHX1B)
SMAD4	Androgen receptor
ESR1 (nuclear)	SMAD4
HIF1A	RelA (p65 NF-kBsubunit)
Androgen receptor	SP1
N-Myc	ESR1 (nuclear)

NFκB as potential regulator *of the DE miRNAs in HER2+ and TN models*

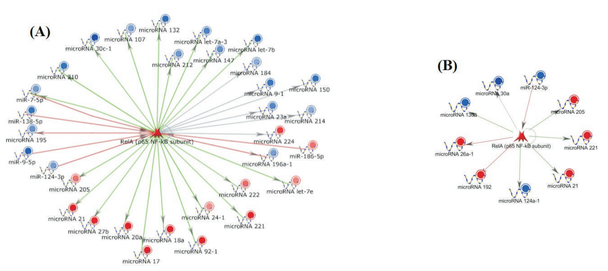
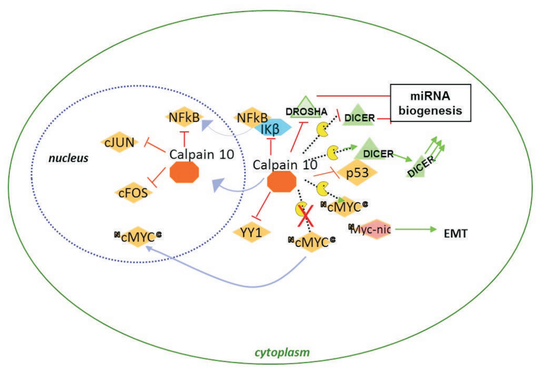


Figure 3: NFκB as potential regulator of DE miRNAs in HER2+ siCAN10 **(A)** and in TN siCAN10 **(B)**.

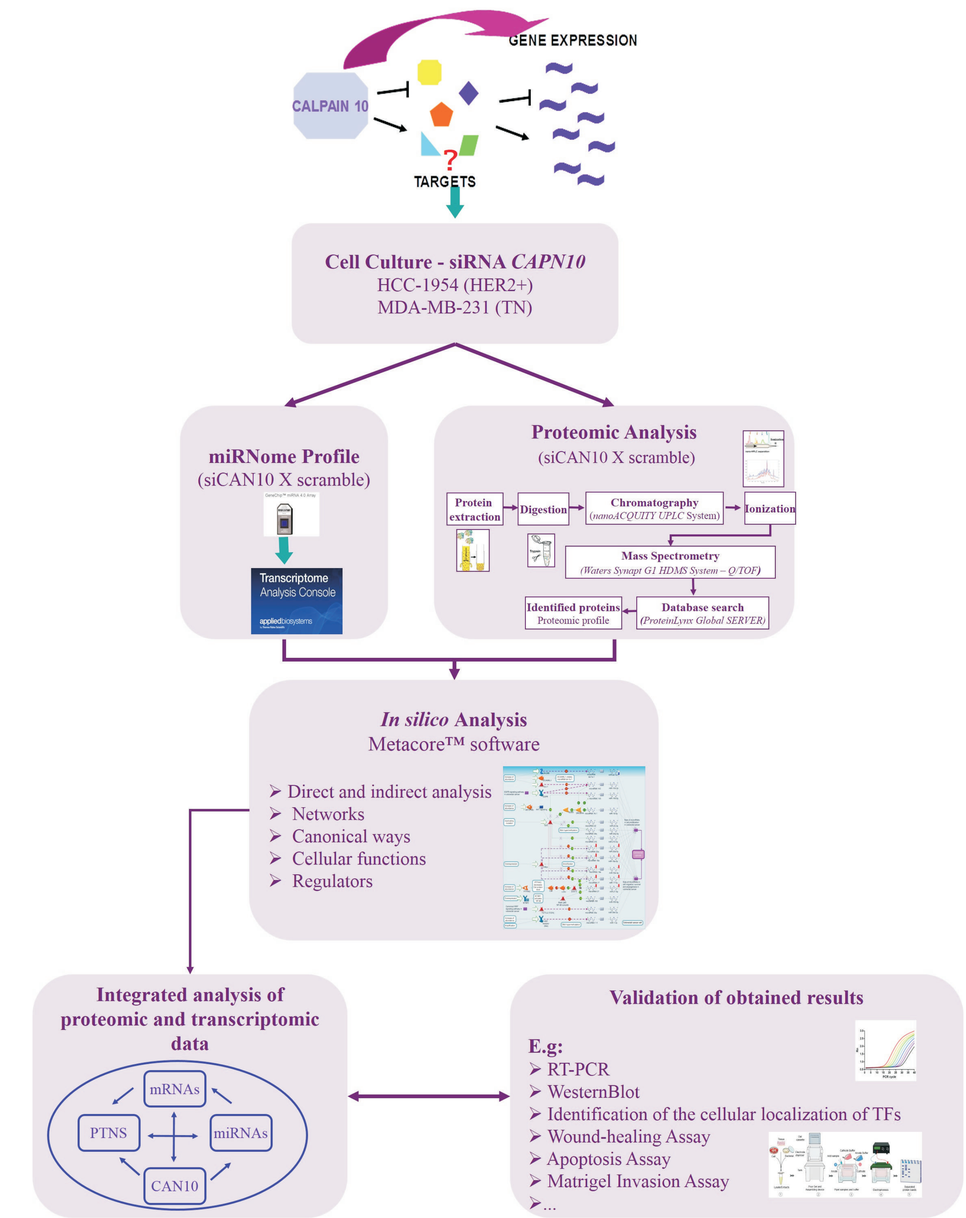
Summary the potential regulation of Calpain 10 in aggressive models of Breast Cancer



OBJECTIVE

The present study aims to investigate the potential role of CAN10 in BC aggressiveness throught genetic and epigenetic mechanisms.

METHODOLOGY



CONCLUSION AND PERSPECTIVES

Our previous data suggest that CAN10 is implicated in BC aggressiveness and may be related with molecular subtypes heterogenety. Integration of direct and indirect targets data together with the investigation of their activation and function will provide evidence of CAN10 role in genetic and epigenetic regulation in BC.

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