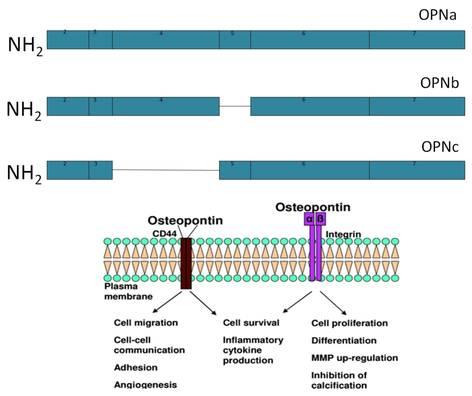
OPNA SPLICE VARIANT OVEREXPRESSION IS ASSOCIATED WITH MATRIX CALCIFICATION AND COLLAGEN DEPOSITION IN THYROID CANCER CELL LINES

Luciana B. Ferreira[1,2], Raquel T. Lima[1], Ana Clara Santos da Fonseca Bastos[2], Andreia Machado Silva[1], Catarina Tavares[1], Ana Pestana[1], Elisabete Rios[1], Catarina Eloy[3], Manuel Sobrinho-Simões[1], Etel Rodrigues Pereira Gimba[2*], Paula Soares[1*]

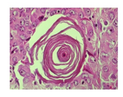
[1]i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [2]National Institute of Cancer, Rio de Janeiro, Brazil; 3Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal. *co-senior authors

BACKGROUND

Osteopontin (OPN) and its three spliced variants (OPN-SV: OPNa, OPNb and OPNc) are overexpressed in several tumors and frequently associated with cancer progression. This holds true for papillary thyroid carcinoma (PTC), which is the most common variety of thyroid cancer (TC), being the histologic type which often presents desmoplasia (collagen deposition) and dystrophic calcification, including a fairly typical feature, the psammoma bodies (PB). This study aims to investigate the OPN-SV expression in classical variant of PTC (cPTC) and the role of OPN in calcification and collagen deposition into the extracellular matrix of thyroid cancer cell lines.



Cancer	Overexpressed isoform	Effects
Prostate	OPNb OPNc	proliferation, migration, invasion
Ovary	OPNc	proliferation, migration, invasion, colony formation
Breast	OPNc	invasion adesion
HCC	OPNa OPNb	migration
Thyoid	OPNa	proliferation, migration, motility, invasion



Psammoma Bodies (PB) – Concentric lamellated calcified structures in PTC

METHODS

Total OPN and OPN-SV expression was analyzed by immunohistochemistry and real time PCR in a series of 48 cPTC cases. The association of OPN expression and the presence of PB as well as between PB in cPTC and the clinicopathological features of the tumors were evaluated. TPC-1 and c643 TC cell lines overexpressing OPN-SV were tested for the ability to promote calcification and to synthesize collagen in vitro. We also evaluated the expression of calcification markers, including osteocalcin and collagen I.

RESULTS

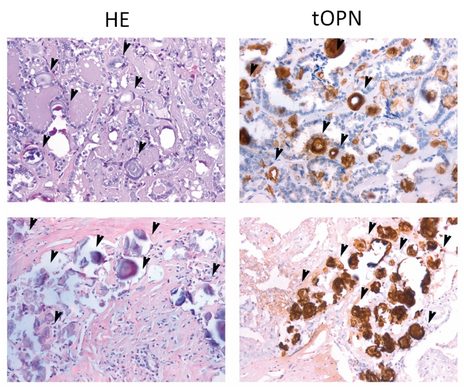


Figure 1. Total OPN staining at PB in cPTC cases. Two different representative cPTC cases showing psammoma bodies (PB) appearing rounded, sometimes fused with each other, or even fragmented, with concentric lamination, as shown by the black arrow heads. PB stained for tOPN antibody, 10 x.

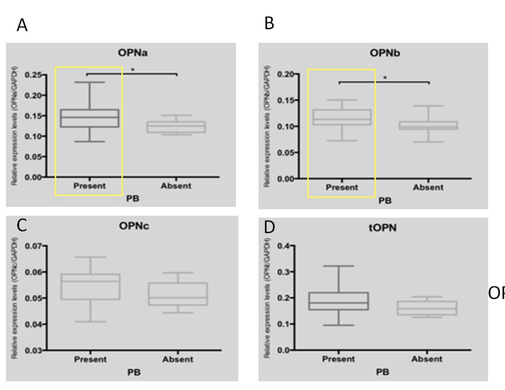


Figure 2. Expression levels of OPNa, OPNb, OPNc and tOPN transcripts in cPTC concerning presence or absence of PB. (A) OPNa (B) OPNb (C) OPNc and (D) tOPN mRNA expression levels measured by real time PCR * $p < 0.05$. Results are from at least two independent assays with triplicates.

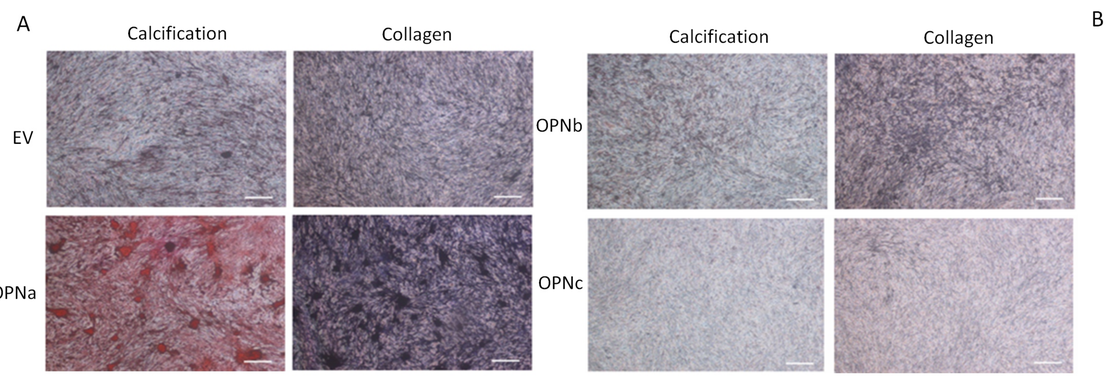


Figure 3. Calcification and collagen production in c643 cells overexpressing OPNa, OPNb, OPNc, EV and TPC1 cells. A) Left panel: Matrix calcification detected with Alizarin Red staining in c643 cell line. Dark orange areas correspond to extracellular matrix (ECM) rich in calcium deposits. Right panel: Collagen ECM production was determined by Masson trichrome staining in c643 cell line. Dark purple areas correspond to ECM rich in collagen. B) Upper: Matrix calcification detected with Alizarin Red staining in TPC1 cell line; Lower: Collagen ECM production was determined by Masson trichrome staining in TPC1 cell line. Scale bar: 100 µM. Representative photomicrographs of 2 independent experiments at 24 days of culture are shown.

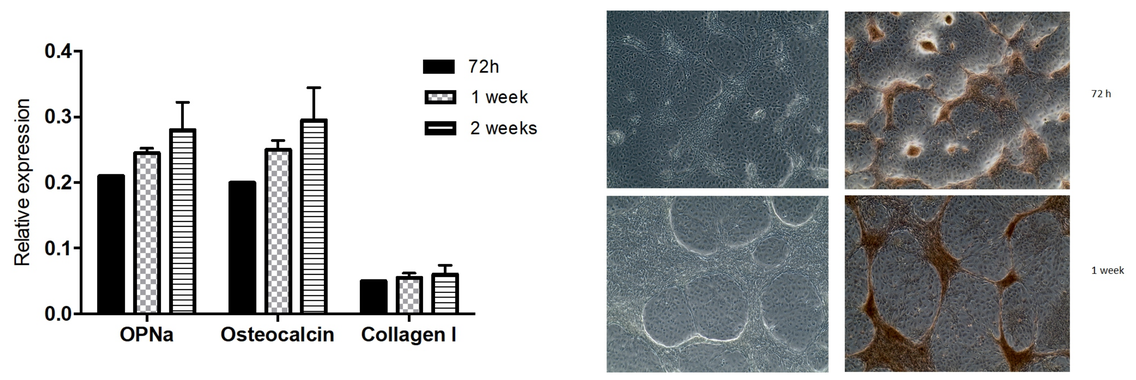


Figure 4. Transcript expression levels of OPNa, osteocalcin and collagen I, and calcification deposits in TPC1 cell line. (A) OPNa, osteocalcin and collagen type I mRNA expression levels has been measured by RTqPCR in TPC1 thyroid cell line after 72 h, 1 and 2 weeks of cell culture. (B) Representative images for 72h and 1 week cell culture of TPC1 cell line are shown at the left column. On the right column, matrix calcification was detected with Alizarin Red staining, after 72 hours and 1 week of cell culture. Dark brown areas correspond to extracellular matrix rich in calcium deposits.

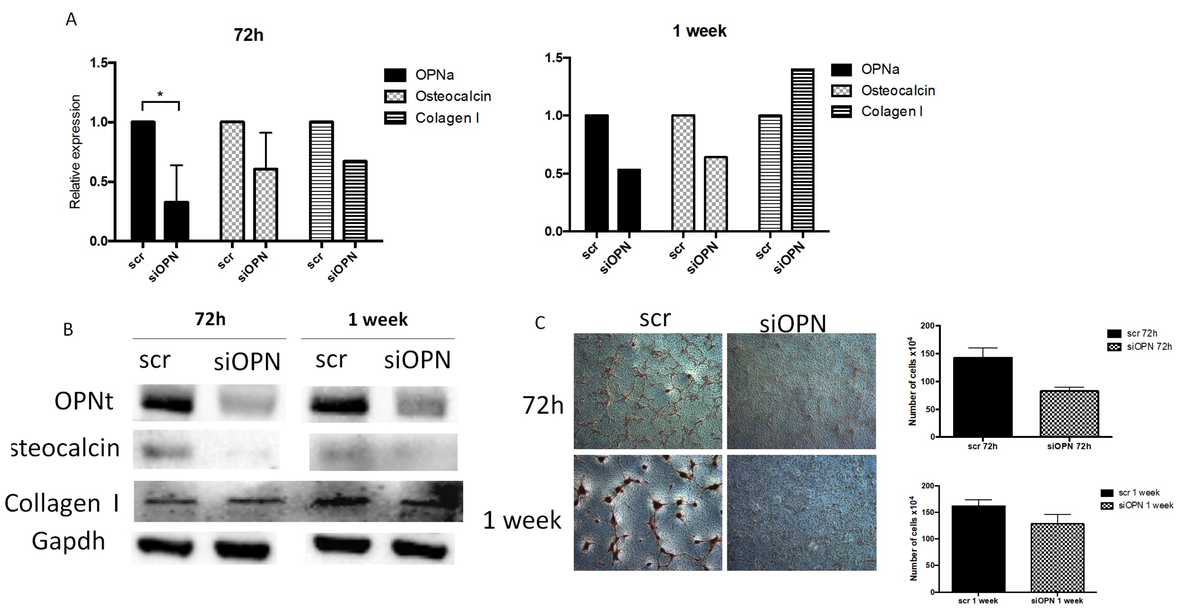


Figure 5. OPNa, osteocalcin, collagen Type 1 mRNA and protein expression, and calcification deposits analyses after OPN silencing. (A) OPNa, osteocalcin and collagen type I mRNA expression levels has been measured by real time PCR in TPC1 thyroid cell line after 72 hours and 1 week of cell culture. Cells were treated with 100nM of siRNA for OPNt and 100nM of siRNA negative control during 72h and 1 week. (B) Western blot was performed for OPN, osteocalcin and collagen type I proteins after OPN silencing. Representative Gapdh expression is shown. Protein level, in treated cells, was evaluated in duplicate. (C) Representative images for 72h after OPN silencing in TPC1 cell line and for the siRNA negative control (scr) are shown. Matrix calcification was detected with Alizarin Red staining, after 72h and 1 week of OPN silencing and the siRNA negative control (scr). Dark orange areas correspond to extracellular matrix rich in calcium deposits. (D) Trypan blue assays showing decreased proliferation levels in TPC1 cells silenced for OPN compared with scrambled negative control after 72 hours and 1 week of knockdown.

CONCLUSIONS

Our data suggest that OPNa plays a role in the extracellular matrix calcification associated with cPTC. Basic research on the interactions between OPNa overexpression by tumor cells and the surrounding microenvironment can give clues for a better understanding of cPTC biology and phenotype.

Funding Norte 2020 – Programa Operacional Regional do Norte, FAPERJ, CNPq, FCT/CAPES e INBEB/CNPq

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