# Osteopontin Modulates Calcification and Matrix Deposition in Thyroid Cancer


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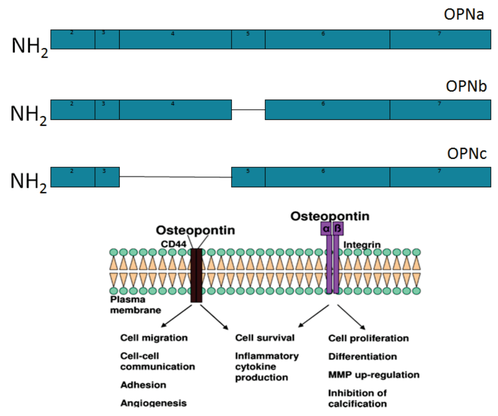
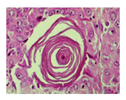
## BACKGROUND

Osteopontin (OPN) and its three splice 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 thyroid cancer (TC) histologic type, often presenting desmoplasia (collagen deposition) and dystrophic calcification, including a fairly typical feature, the psammoma bodies (PB). The aim of this study was to investigate the role of OPN-SV on modulating calcification processes in the classical variant of PTC (cPTC). The aim of this study was to investigate the role of OPN-SV expression in the development of PB in classical variant of PTC (cPTC).




| Cancer | Overexpressed isoform | Effects |
|---|---|---|
| **Prostate** | OPNb<br>OPNc | proliferation, migration, invasion |
| **Ovary** | OPNc | proliferation, migration, invasion, colony formation |
| **Breast** | OPNc | invasion<br>adesion |
| **HCC** | OPNa<br>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 and three diffuse sclerosing PTCs. 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*.

## RESULTS

Overexpression of OPNa transcripts was significantly associated with the presence of PB in cPTC samples. The presence of PB in cPTC was associated with younger patients and lymph node metastasis. Moreover, OPNa overexpression displayed a strong capacity to promote calcification and substantial collagen synthesis in thyroid cancer cell lines. In addition, when total OPN was suppressed by siRNA approach, calcification was reduced, as well as calcification markers, including osteonectin and collagen type I. Our data suggest that OPNa plays a role in the calcification process, often 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.

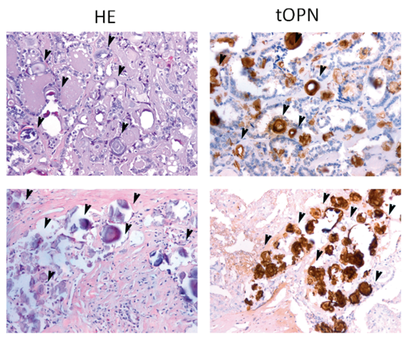



Figure 1. Total OPN staining at PB in cPTC cases. Two different representative cPTC cases showing psammoma bodies (PB) appearing rounded, sometimes fused with each other, or even fragmented, with concentric lamination, as shown by the black arrow heads. PB stained for tOPN antibody, 10 x.

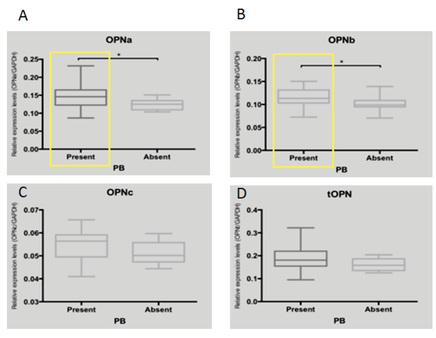



Figure 2. Expression levels of OPNa, OPNb, OPNc and tOPN transcripts in cPTC concerning presence or absence of PB. **(A)** OPNa **(B)** OPNb **(C)** OPNc and **(D)** tOPN mRNA expression levels measured by real time PCR * $p < 0.05$. Results are from at least two independent assays with triplicates.

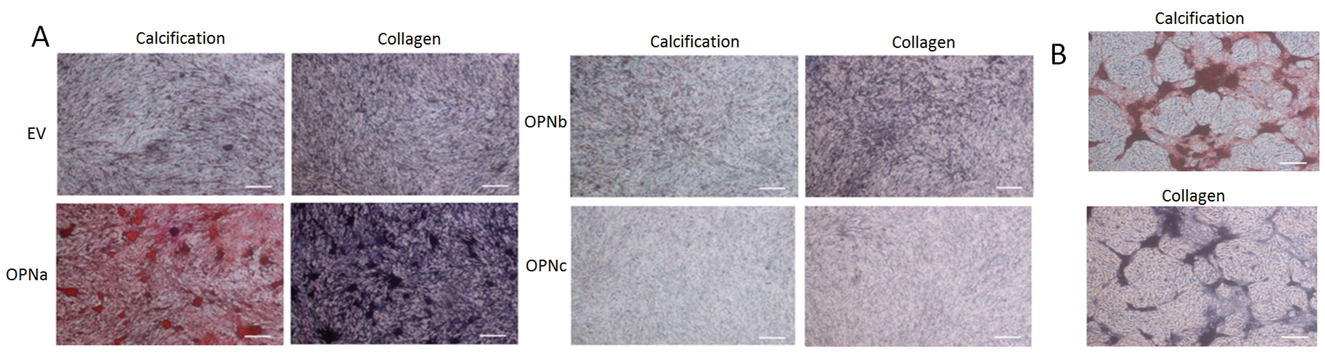



Figure 3. Calcification and collagen production in c643 cells overexpressing OPNa, OPNb, OPNc, EV and TPC1 cells. A) Left panel: Matrix calcification detected with Alizarin Red staining in c643 cell line. Dark orange areas correspond to extracellular matrix (ECM) rich in calcium deposits. Right panel: 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.

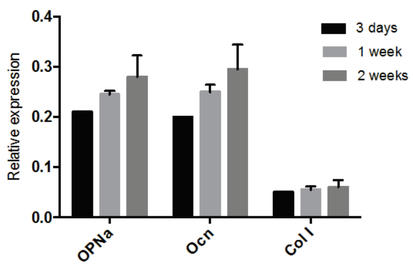



Figure 4. Expression of OPNa, Osteocalcin (Ocn) and Colagen I (Col I) mRNA in TPC1 thyroid cell line. Expression of OPNa, Ocn and Col I by RT-qPCR in TPC1 thyroid cancer cell line cultured in 3 days, one and two weeks. Expression of OPNa, Ocn and Col I are presented relative to GAPDH gene expression.

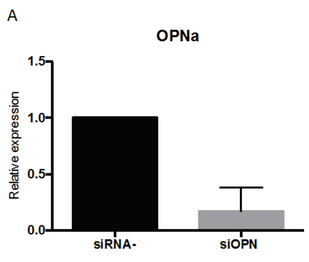




Figure 5. OPN expression levels in TPC1 cells silenced for osteopontin mRNA. A) OPNa gene expression analysis by RT-qPCR indicating OPN suppression compared to siRNA control. B) Western blot analysis indicating reduced amount of OPN protein after OPN mRNA silencing. Gapdh was used as a loading control.

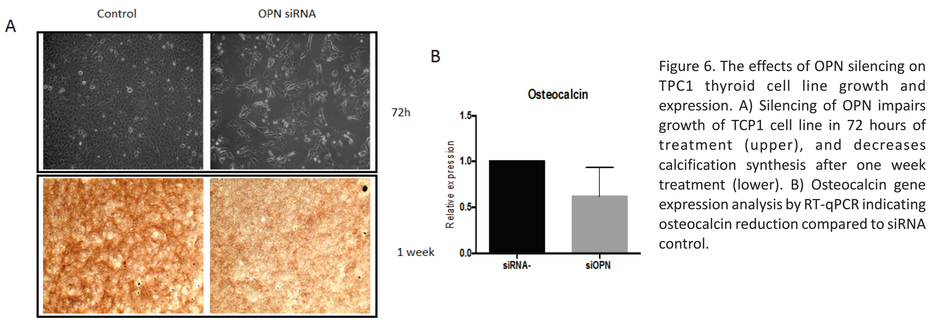



Figure 6. The effects of OPN silencing on TPC1 thyroid cell line growth and expression. A) Silencing of OPN impairs growth of TCP1 cell line in 72 hours of treatment (upper), and decreases calcification synthesis after one week treatment (lower). B) Osteocalcin gene expression analysis by RT-qPCR indicating osteocalcin reduction compared to siRNA control.

## CONCLUSIONS

Our data suggest that OPNa plays a role in the formation of PB often associated with cPTC. Also, OPN expression levels seems to modulate the matrix mineralization process. Basic research on the interactions between OPNa overexpression by tumor cells and the surrounding microenvironment may give clues for a better understanding of cPTC biology and phenotype.


**Key-words:** osteopontin; thyroid cancer; calcification

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