OSTEOPONTIN-A SPLICE VARIANT MODULATES MEDULLARY THYROID CARCINOMA CELL DIFFERENTIATION FEATURES

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INTRODUCTION

Medullary thyroid carcinoma (MTC), which originates from thyroid C-cells, accounts for 1 to 2% of thyroid tumors. Although rare, MTC has a high level of malignancy, due to the high incidence of metastasis. Osteopontin (OPN) is a glycoprotein overexpressed in several tumor types and generates by alternative splicing at least the variants OPNa, OPNb, OPNc, OPN4 and OPN5. OPNa is the most expressed variant in MTC and has been related to cell differentiation and good prognosis in this tumor type.

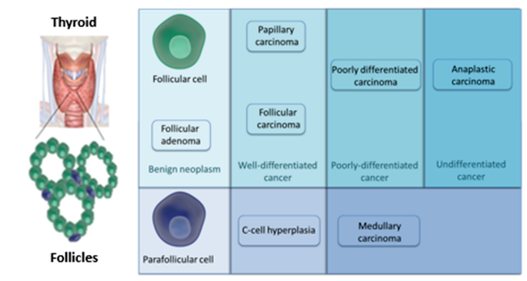


Figure 1: Different types of thyroid cancers derived from the two cellular types of this gland: follicular cells and parafollicular cels or C-cells (Adapted from Ferreira, L.B., 2016).

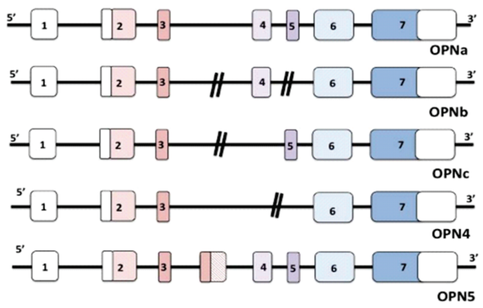


Figure 2: The five OPN splice variants. The OPNa variant represents the canonical isoform, containing the 7 exons, while OPNb lacks exon 5 and OPNc lacks exon 4. The OPN4 variant lacks exons 4 and 5 and OPN5 has an extra exon generated by part of intron's 3 retention and a different initiation codon comparing to the other variants (Bastos et al., 2017).

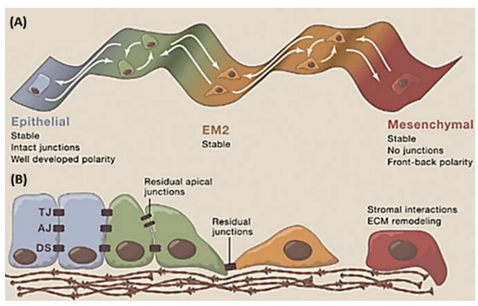
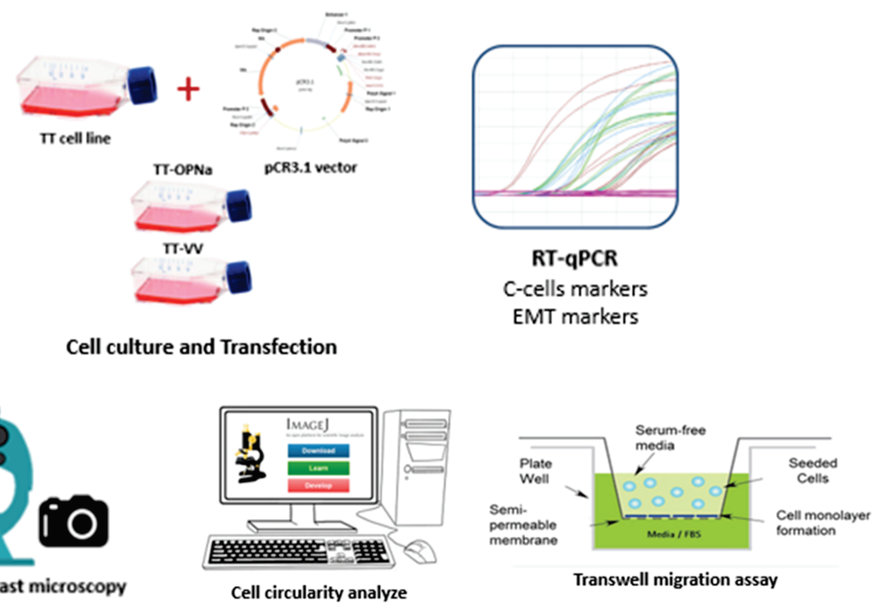


Figure 3: Representation of epithelial-mesenchymal transition (EMT) process. (A) The ripples and the white arrows demonstrate the plasticity that often occurs in EMT, from a spectrum of intermediate phases, where there may be great variation in the phenotype and progression stages. (B) Cells moving from an epithelial phenotype (blue cells) to a mesenchymal phenotype (red cells), passing through intermediate stages (green and yellow cells). TJ: Tight junctions; AJ: adherens junctions; DS: desmossome (Adapted from Nieto et al., 2016).

OBJECTIVES

Based on these data, the aim of this work was to better understand the role of the OPNa variant on modulating MTC differentiation, correlating its expression with C-cells specific differentiation markers and EMT features.

METHODOLOGY



CONCLUSIONS

- OPNa's overexpression in TT cell line, derived from MTC, promoted increase in expression of C-cells differentiation and epithelial markers, as well as decrease in expression of mesenchymal markers.
- TT-OPNa cells also showed epithelial cell-morphological features, greater cell circularity and fewer migratory cells.
- These data corroborate previous findings relating OPNa variant to good prognosis characteristics in the MTC model.

RESULTS

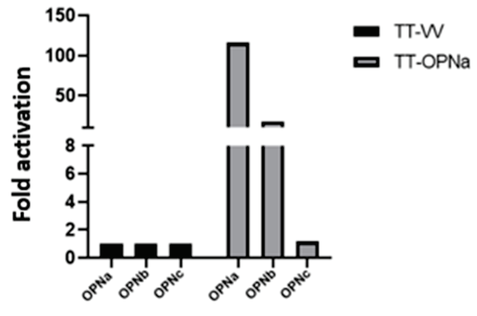


Figure 4: Expression levels of OPNa, OPNb and OPNc splice variants by RT-qPCR in TT-OPNa and TT-VV cell lines. Relative transcriptional expression levels of OPNa, OPNb and OPNc genes were evaluated by RT-qPCR, in duplicate at one single assay, using specific oligonucleotides and *GAPDH* as the housekeeping gene. The TT-VV cell line was used as the reference samples (Reference value = 1).

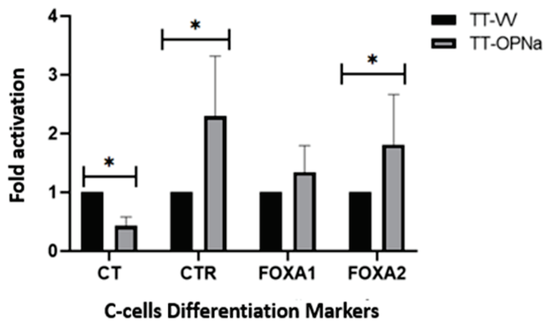


Figure 5: Transcriptional levels of C-cells differentiation markers in response to OPNa overexpression. The relative expression levels of *CT, CTR, FOXA1* and *FOXA2* genes were evaluated by RT-qPCR, using specific oligonucleotides and *GAPDH* as the housekeeping gene. The results were analyzes in duplicate at three independent assays TT-VV cell line was used as the reference samples (Reference value = 1). *P < 0.05.

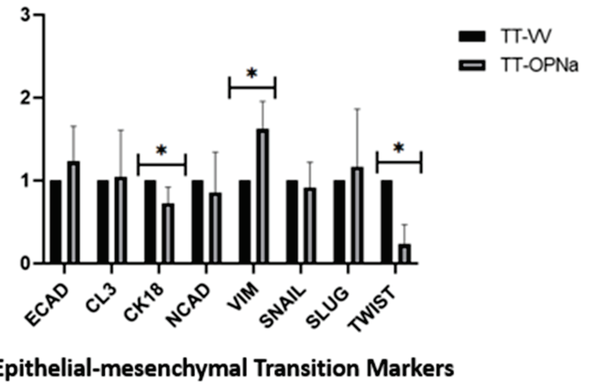


Figure 6: Transcriptional levels of the EMT markers in response to OPNa overexpression. The relative expression levels of *E-cadherin, Claudin3, Cytokeratin18, N-cadherin, Vimentin, Snail, Slug* and *Twist* genes were evaluated by RT-qPCR using specific oligonucleotides and *GAPDH* as the housekeeping gene. The resulsts were analyzes in duplicate at three independent assays. TT-VV cell line as used as the reference samples (Reference value = 1). *P < 0.05.

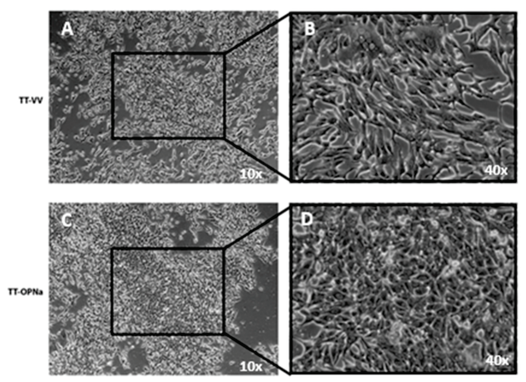


Figure 7: TT-OPNa cells presented epithelial features. Cell morphology analysis and aggregational state of TT-VV and TT-OPNa cell lines. (A and B) TT-VV cells. (C and D) TT-OPNa cells. (A and C) Objective magnification: 10x (B and D) Objective magnification: 40x.

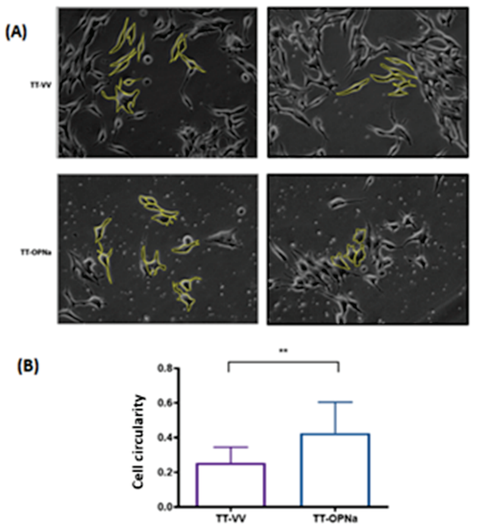


Figure 8: OPNa overexpression increased cell circularity. TT-VV and TT-OPNa cell circularity were evaluated using the ImageJ software, with the 4π x [Area]/[Perimeter]^2 formula. (A) The yellow lines represent how the circularity was measured, in which each cell of a representative field was surrounded, generating a value from 0 to 1. (B) Representative graph of TT-VV and TT-OPNa cell circularity measurement. Objective magnification: 10x. **P=0.0016.

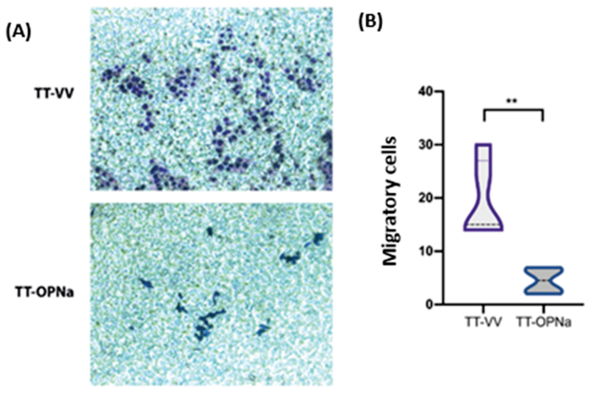


Figure 9: OPNa overexpression inhibited cell migration. (A) TT-VV and TT-OPNa cell migration were represented by pictures stained membrane's representative fields. (B) The number of migratory cells was represented by graph. In one single assay, 5 stained membrane fields were photographed, the cells were counted and its total number plotted in graph. **P = 0.0079.

KEYWORDS: medullary thyroid carcinoma; osteopontin; cell differentiation.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA