THE RECENTLY DESCRIBED OSTEOPONTIN-4 AND OSTEOPONTIN-5 ARE EXPRESSED IN SEVERAL TUMOR CELL LINES

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KEYWORDS: Osteopontin, Osteopontin splice variants, OPN-4, OPN-5.

INTRODUCTION AND OBJECTIVES

- Osteopontin (OPN) is an extracellular matrix phosphoglycoprotein and is overexpressed in several tumor types, thus contribute to oncogenesis and tumor progression.
- The OPN coding gene contains 7 exons and undergoes *alternative splicing* (AS) resulting in 5 variants, called OPN-a, OPN-b and OPN-c. Recently two other isoforms have been described, OPN-4 and OPN-5.
- The present work aimed to investigate the expression of OPN-4 and OPN-5 isoforms in several cancer cell lines, besides evaluating their expression levels in relation to previously described OPN *splicing* variants (OPN-SVs).

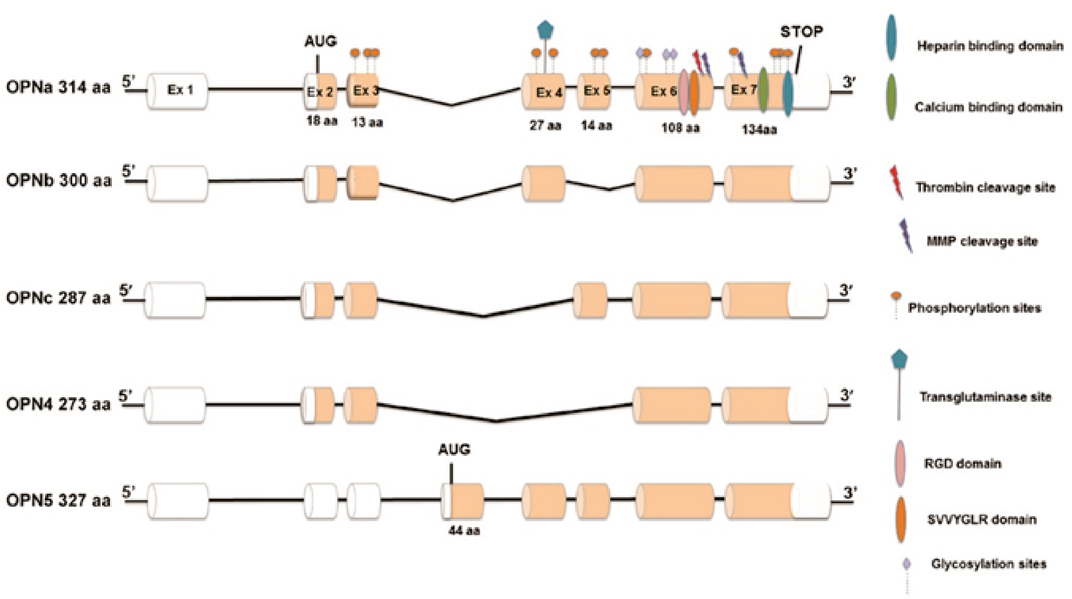

Figure 1: OPN-SVs and their respective exon arrangements. The complete isoform, denominated OPN-a, contains 7 exons represented by white (non-coding) and color (coding) boxes. OPN-b and OPN-c do not have exons 5 and 4, respectively, while OPN-4 does not have exons 4 and 5. On the other hand, OPN-5 contains an additional exon (hatched box) located between exons 3 and 4, which results from the inclusion of part of intron 3. Retirado de Gimba et al, 2019

MATERIAL AND METHOD

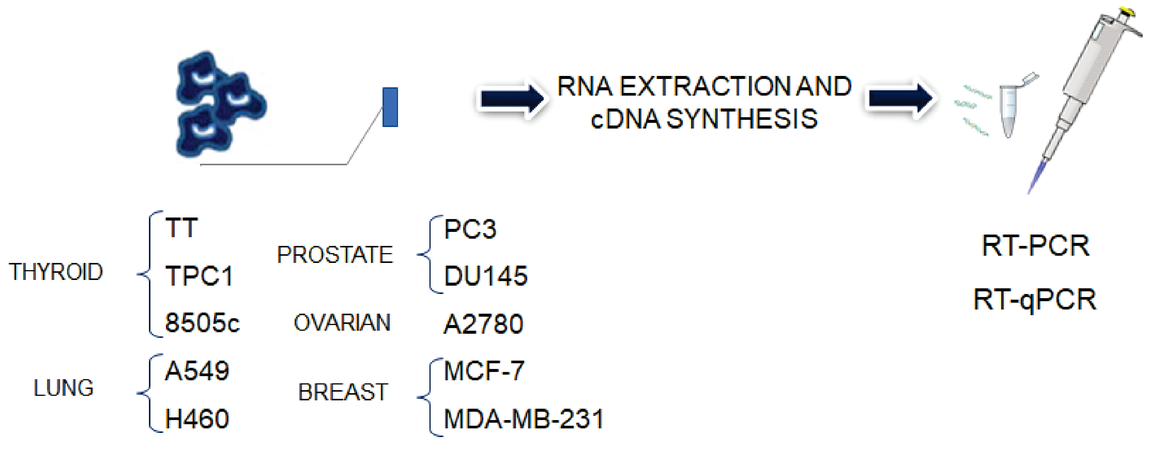

Figure 2: Total RNA from cancer cell lines have been extracted, followed by cDNA synthesis. OPN-SVs transcript analysis has been performed using isoform-specific oligonucleotides and GAPDH and actin genes were used as normalization controls. Student's T and Mann-Whitney tests were used for statistical analysis.

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RESULTS

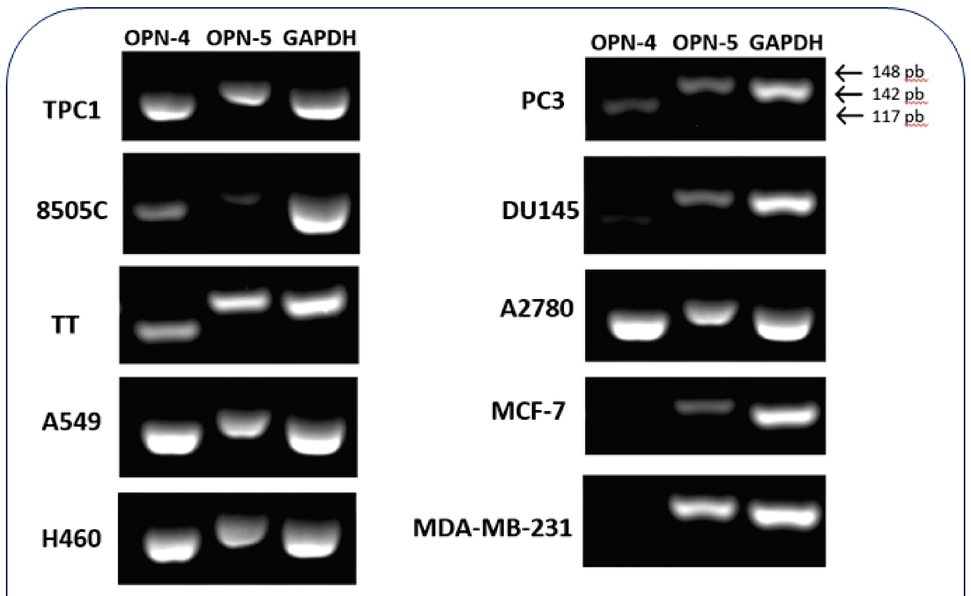

Figure 3: Qualitative expression profile of OPN isoforms in tumor cell lines. Qualitative expression of OPN isoform transcripts was obtained using from RT-PCR assays and amplification products were analyzed by horizontal electrophoresis by agarose gels. Amplification product sizes: OPN-4 = 117 bp, OPN-5 = 148 bp and GAPDH = 142 bp.

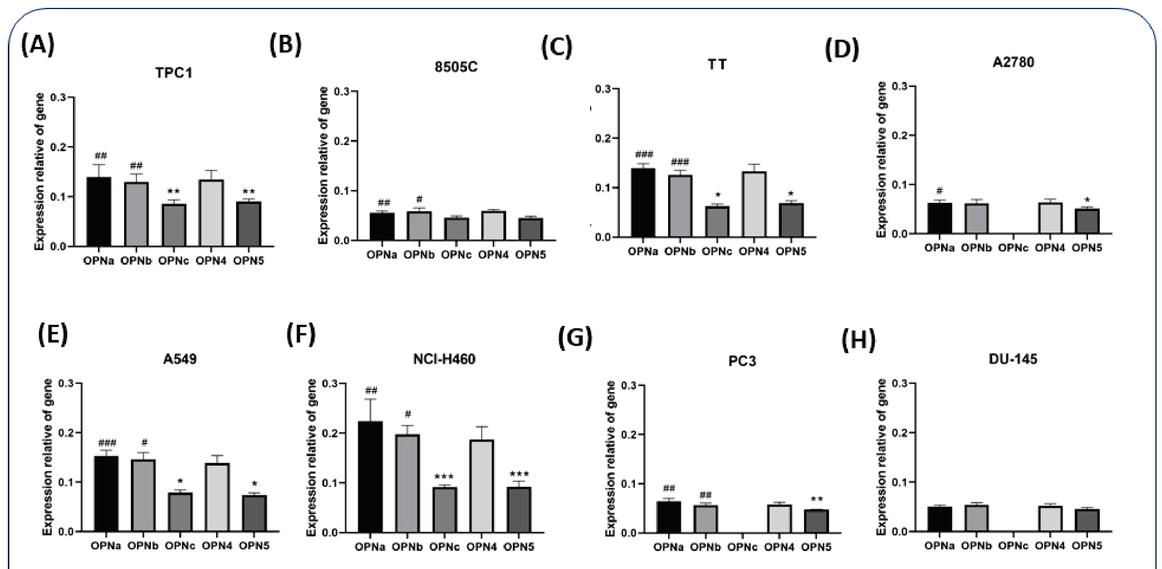

Figure 4: Quantitative expression profile of OPN isoforms in tumor cell lines. For amplification of the transcripts of the OPN-a, OPN-b, OPN-c, OPN-4 and OPN-5 isoforms, specific oligonucleotides for these isoforms were used, with GAPDH and β-actin being the constitutive expression gene. The results presented were performed in duplicate and in 3 independent assays. **(A) (B) (C)** Relationship between transcriptional levels of isoforms OPN-a, OPN-b, OPN-c, OPN-4 and OPN-5 in thyroid tumor cell lines, **(D)** Relationship between transcriptional levels of OPN-a, OPN-b, OPN-c, OPN-4 and OPN-5 isoforms in ovarian tumor cell lines, **(E) (F)** Relationship between transcriptional levels of OPN-a, OPN-isoforms b, OPN-c, OPN-4 and OPN-5 in lung cancer cell line, (G) (H) Relationship between transcriptional levels of the OPN-a, OPN-b, OPN-c, OPN-4 and OPN-5 in prostate tumor cell lines. The asterisks (*) refer to the comparison between the transcriptional expression level of the OPN-a, OPN-b, OPN-c and OPN-5 isoforms in relation to the OPN-4 isoform in the different strains. While the bullet (#) refers to the comparison between the transcriptional expression level of the OPN-a, OPN-b, OPN-c and OPN-4 isoforms relative to the OPN-5 isoform. * p <0.05, ** p <0.01 and *** P <0.001; #p <0.05, ## p <0.01 and ### p <0.001 Student's t test or Mann-Whitney.

CONCLUSIONS

OPN-4 and OPN-5 variants, similarly the other previously described OPN-SV, seems to be expressed in several tumor types. Furthermore, according to their expression patterns in relation to OPN-a, OPN-b and OPN-c isoforms, we then hypothesize that they may also contribute to key aspects of tumor progression and should be further investigated in each specific tumor model.

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