EXPRESSION PROFILE AND FUNCTIONAL ROLE OF SPLICING ISOFORMS OF OSTEOPONTIN IN COLORECTAL CARCINOMA CELLS

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INTRODUCTION

Osteopontin (OPN) is an extracellular matrix protein overexpressed in several tumor types, including colorectal carcinoma (CRC). The OPN primary transcript undergoes alternative splicing, generating at least five OPN splicing variants (OPN-SVs), named OPNa, OPNb, OPNc, OPN4 and OPN5. Although total OPN performs known roles in CRC tumor progression and has been cited as biomarker for this tumor, to date the expression profile and functions of these distinct isoforms in CRCs has not been addressed.

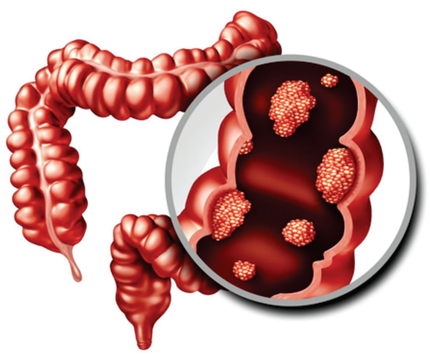


Figure 1. Colorectal cancer illustrative image

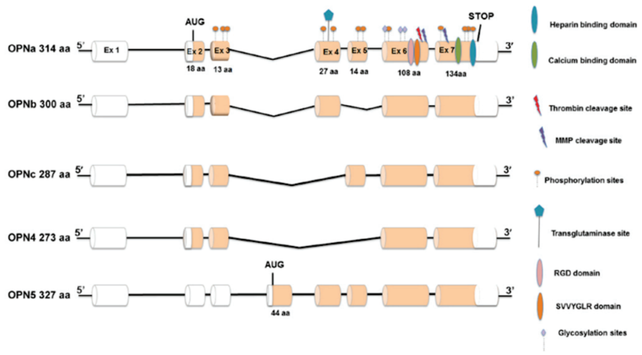


Figure 2. OPN-SVs generated by alternative splicing. (Gimba et al., 2019)

OBJECTIVES

This study aims to characterize the expression profile and the functional roles of OPN-SV in CRC cells.

METHODOLOGY

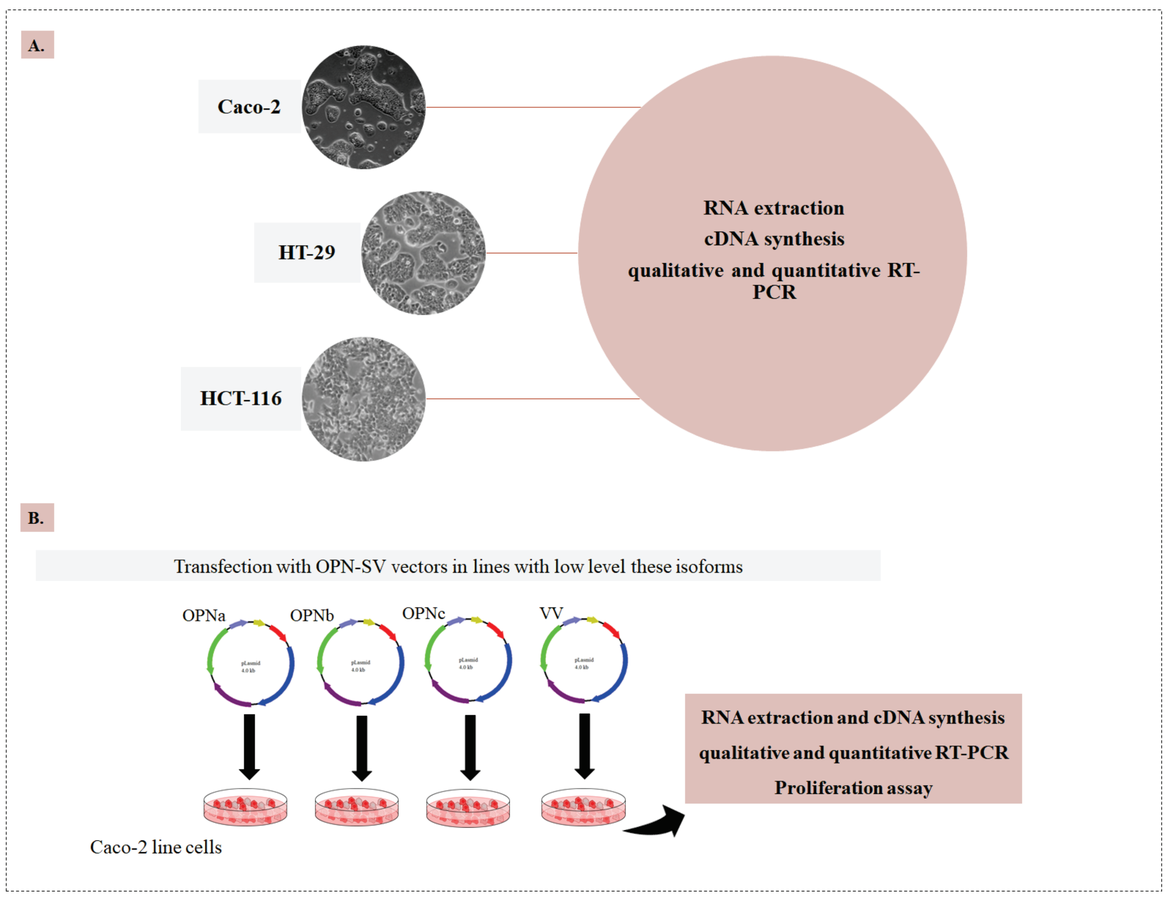


Figure 3. Experimental approach. Quantitative and qualitative RT-PCR assays have been performed in order to analyze the expression of OPN-SV in CRC tumor cell lines (Caco-2, HT-29 and HCT-116). Caco-2 cell line was transfected with expression vector containing the complete cDNA of OPNa, OPNb or OPNc or with empty vector (VV) control plasmid. Proliferation assays have been performed by trypan blue exclusion assays.

RESULTS

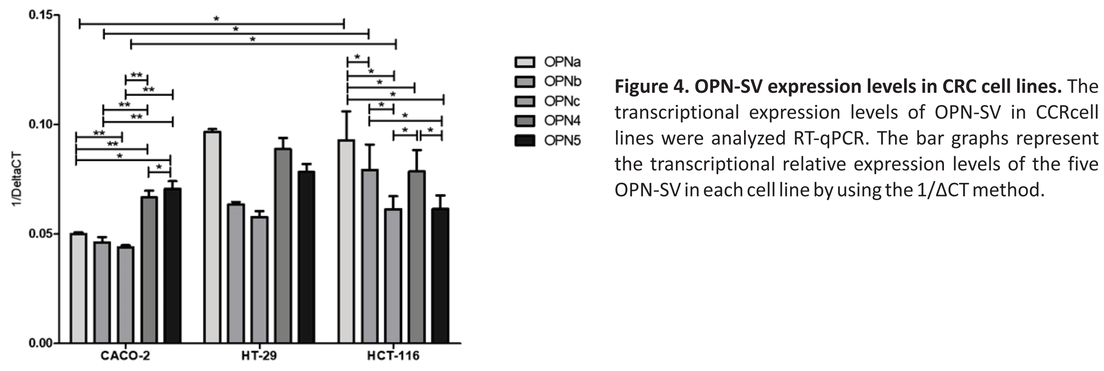
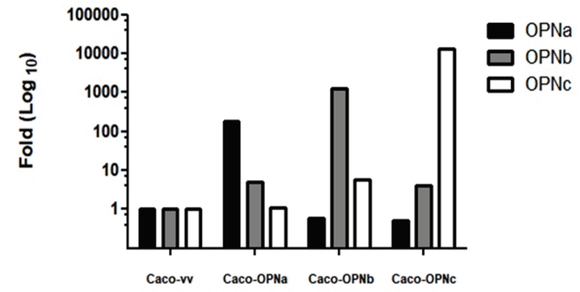


Figure 4. OPN-SV expression levels in CRC cell lines. The transcriptional expression levels of OPN-SV in CCRcell lines were analyzed RT-qPCR. The bar graphs represent the transcriptional relative expression levels of the five OPN-SV in each cell line by using the 1/ΔCT method.

Figure 5. OPN-SV expression levels in transfected Caco-2 cells. The transcriptional expression levels of OPN-SV in Caco-2 heterogeneous cell clones stably transfected with OPNa, OPNb, OPNc, or with in empty expression vector control (VV) were analyzed RT-qPCR. The bar graphs represent the relative expression at transcriptional level of the three isoforms of the OPN from analyses of $2^{-\Delta\Delta CT}$.



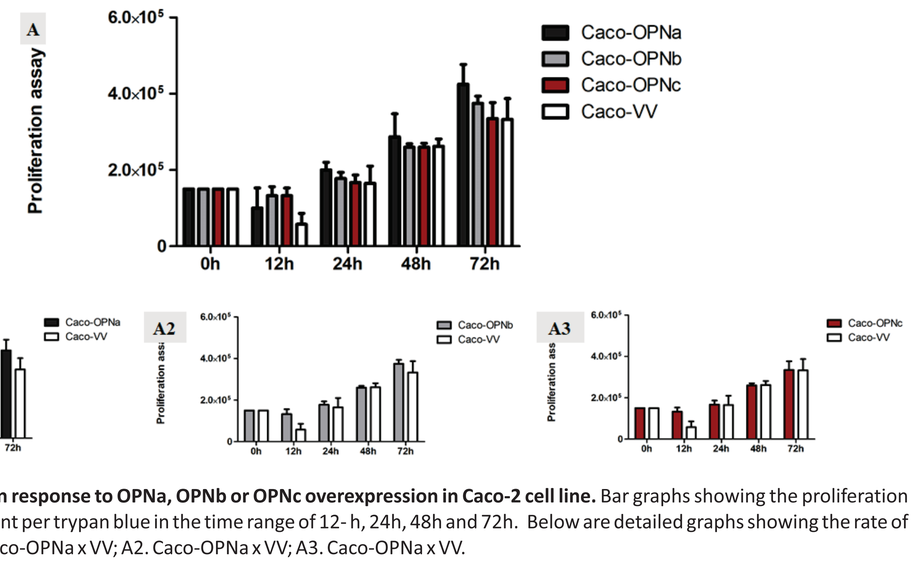


Figure 6. Proliferation rates in response to OPNa, OPNb or OPNc overexpression in Caco-2 cell line. Bar graphs showing the proliferation rates according to the cell count per trypan blue in the time range of 12- h, 24h, 48h and 72h. Below are detailed graphs showing the rate of proliferation between: A1. Caco-OPNa x VV; A2. Caco-OPNa x VV; A3. Caco-OPNa x VV.

CONCLUSIONS

These data suggest that these OPN-SVs are co-expressed in CRC tested cell lines and that overexpressed OPNa may indicate its association with CRC undifferentiated phenotypes. Based on the differential expression of OPN-SVs among CRC cell lines, we provide early evidence that OPN-SVs are under transcriptional control, as in other tumor types. We observed Caco-2 cells transfected with OPNa showed a higher proliferative rate than the other two isoforms, suggesting that this OPN-SV could influence tumor growth. Further functional studies will be performed with clones ectopically expressing these OPN-SVs in order to better comprehend their roles in CCR biology.

KEYWORDS: Osteopontin, colorectal cancer cell carcinoma, splice variants

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