OSTEOPONTIN-C ACTIVATES B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PROGRESSION FEATURES

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INTRODUCTION

Osteopontin (OPN) primary transcript is subject to alternative splicing, generating at least five OPN splicing isoforms (OPN-SI) named OPNa, OPNb, OPNc, OPN4, and OPN5. A hallmark of B- cell acute lymphoblastic leukemia (B-ALL) is the occurrence of specific gene rearrangements associated with prognostic risk stratification. Patients harboring the *KMT2A-AFF1* gene fusion are included in the poor prognostic subgroup, with an overall survival rate of 35% and increased risk of extramedullary sites involvement. In B-ALL, it has been reported that total OPN (tOPN) supports tumor dormancy and cell survival in response to chemotherapeutic drugs. However, the putative roles of OPN-SI in B-ALL cells have not been addressed. Previous data from our group showed that OPNc is overexpressed in B-ALL patients harboring the *KMT2A-AFF1* gene fusion and is correlated to poor prognostic features (Bastos *et al.*, 2019 sob revisão).

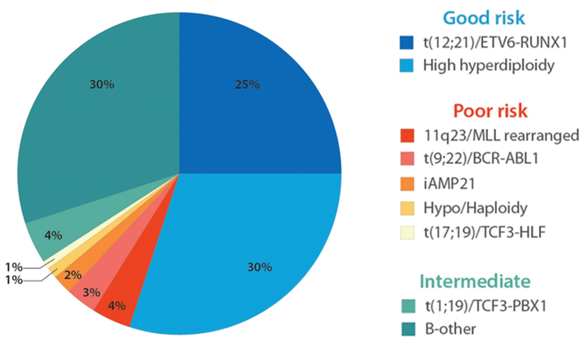


Figure 1: Frequency of molecular-cytogenetic abnormalities in B-ALL. Rearrangements of the *KMT2A* (also known as mixed lineage leukemia (MLL) gene located on chromosome 11q23 are observed in 4% of ALL patients and are related to poor prognosis. (Adapted from Schwab & Harrison, 2018)

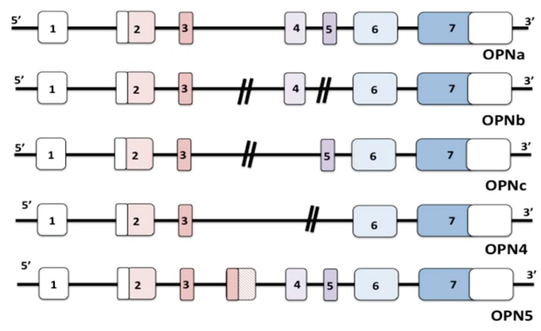


Figure 2: OPN gene structure, described splice variants and their respective exon arrangements. The full length isoform, named OPNa, contains 7 exons represented by white (non-coding) and colored (coding) boxes. While OPNb and OPNc lack exons 5 and 4, respectively (Bastos et al., 2017).

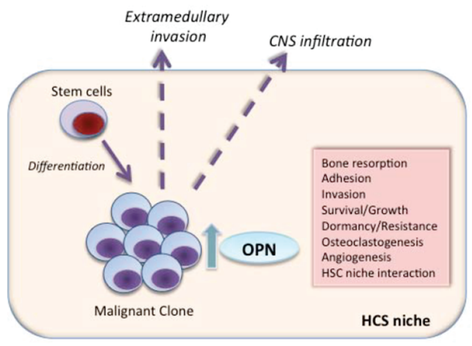


Figure 3: OPN perform distinct roles in HMs. In the bone marrow (BM) niche, upregulated OPN secreted by malignant clones, as well as by additional cells present in the BM microenvironment (e.g. osteoblasts) can promote differentiation of hematopoietic progenitors and stem cells. Overexpressed OPN can then induce several steps elated to HMs progression, such as cell adhesion, invasion, tumor growth, cell survival, dormancy, angiogenesis and osteoclatogenesis. Each of these tumor features mostly occur depending on each type of HM. In response to OPN overexpression, tumor cells can also invade or infiltrate other tissues, such as extramedullary sites and central nervous system (CNS) (Bastos et al., 2017).

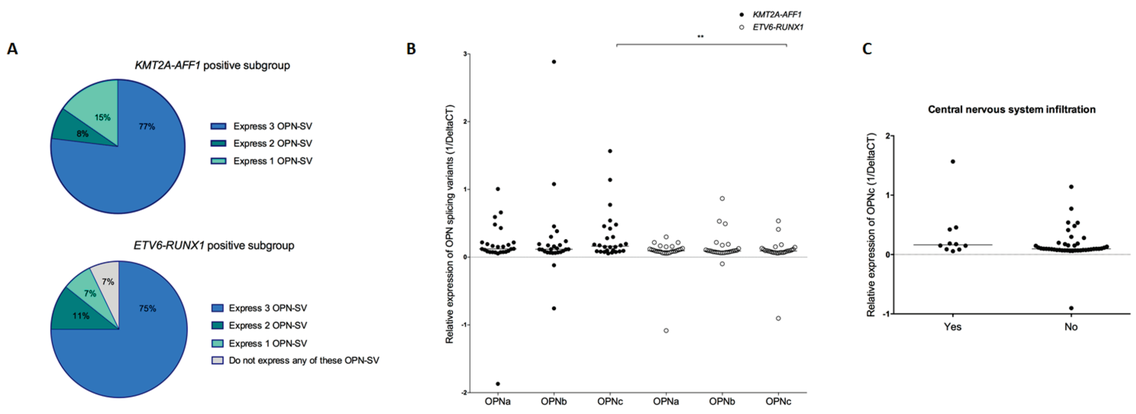


Figure 4: Frequency and expression levels of OPN-SIs in B-ALL patient samples. (A) Frequency of OPN-SIs expression levels in B-ALL patient samples. The frequency of OPN-SIs expression levels in B-ALL patients subgroups with *KMT2A-AFF1* fusion gene or ETV6-RUNX1 rearrangement, as a comparative group. **(B)** The mRNA expression levels of OPN-SIs in B- ALL patient samples were analyzed using real-time RT- PCR. Dot-plot graph represents OPN-SI relative expression levels as demonstrated by 1/Delta CT. Actin gene has been used as the reference gene. OPNc and OPNa isoforms are expressed in higher levels in patients samples harbouring either *KMT2A-AFF1* or ETV6-RUNX1 rearrangements. Additionally, patients harbouring *KMT2A- AFF1* fusion exhibit higher OPNc transcriptional levels those harbouring ETV6-RUNX1 rearrangement (p=0.0056). **(C)** Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients *KMT2A-AFF1* positive with CNS infiltration present higher median OPNc transcriptional expression levels than those patient samples without CNS infiltration.

OBJECTIVES

This study aimed to investigate the putative roles of OPNc on modulating cellular and molecular aspects of B-ALL cells with *KMT2A-AFF1.*

METHODOLOGY

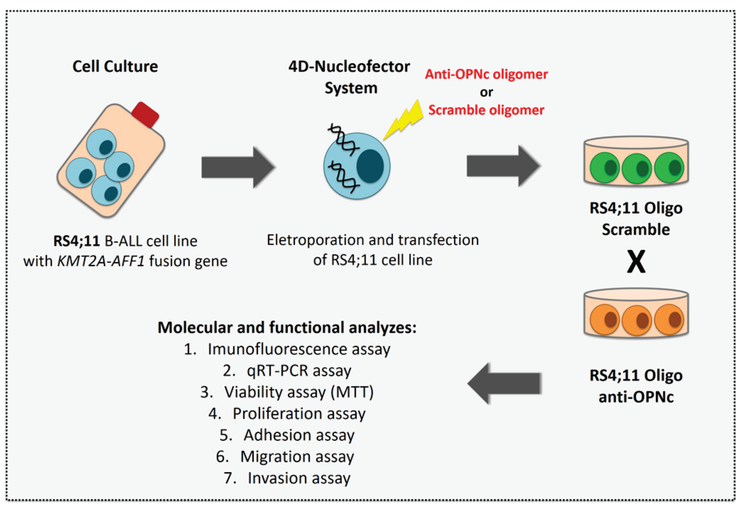


Figure 5: Methodology. RS4;11 B-ALL cell line with KMT2A-AFF1 fusion gene was transfected with anti-OPNc oligomers or scramble oligomers using 4D-Nucleofector System. Cells transfected with anti-OPNc oligomer was named RS4;11 Oligo anti-OPNc and cell transfected with the scramble oligomer was named RS4;11 Oligo scramble. Molecular and functional analysis such as qRT-PCR, cell viability, proliferation, adhesion, migration and invasion assays were performed in response to OPNc silencing.

RESULTADOS

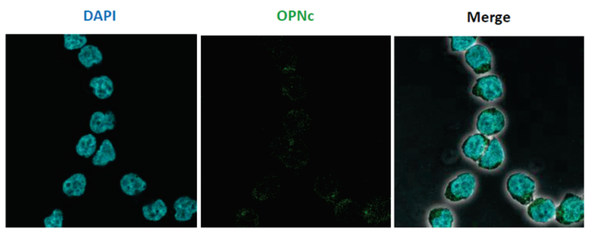


Figure 6: OPNc expression levels in RS4;11 cell line. The expression of OPN-c in RS4;11 cell line was analyzed by immunofluorescence assay. Cells were incubated in the presence of OPNc antibody conjugated with FITC and DAPI. Imagens were analyzed using confocal laser microscopy.

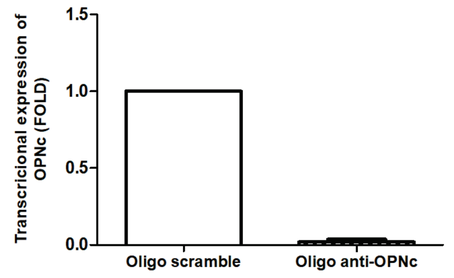


Figure 7: OPNc was efficiently knocked-down using anti-OPNc antisense DNA oligomers modified with phosphorothiotates. The RS4;11 B-ALL cell line was transfected using 50 nM anti-OPNc or scrambled DNA oligomers by the 4D- Nucleofector.

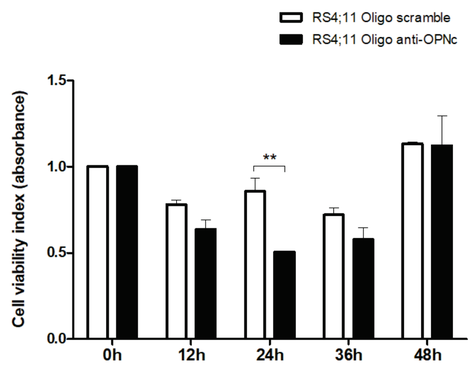


Figure 8: OPNc knock-down inhibit RS4;11 B-ALL cell viability rates. Curve graphs represent viability rates according to MTT analysis every 12 hours in cells transfected with 50nM of anti-OPNc or scrambled DNA oligomers at 0-48h time range. Cell viability rates were evaluated by absorbance measurement at 650nm (p=0.009).

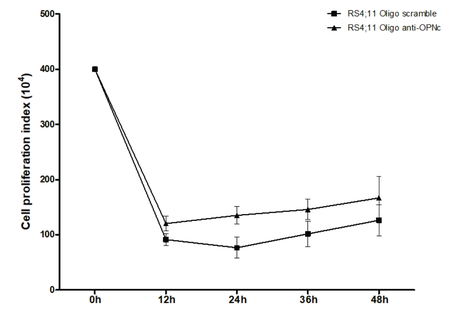


Figure 9: OPNc knock-down inhibit RS4;11 B-ALL cell proliferation rates. The RS4;11 B-ALL cell line exhibit increased proliferation rates in response to OPNc silencing at 12-48 h post transfection with anti-OPNc oligomers. Curve graphs represent proliferation rates according to trypan blue cell counting analysis every 12 h in cells transfected with 50nM of anti-OPNc or scrambled DNA oligomers at 0-48 h time range. Cell number were determined by Trypan blue.

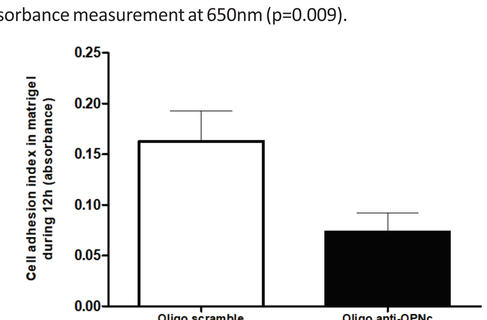


Figure 10: OPNc knock-down inhibit RS4;11 B-ALL cell adhesion rates over matrigel. Bar graphs represent cell adhesion rates over matrigel. Cells were transfected with 50nM of anti-OPNc or scrambled DNA oligomers. Cell adhesion rates were evaluated by absorbance measurements at 650nm

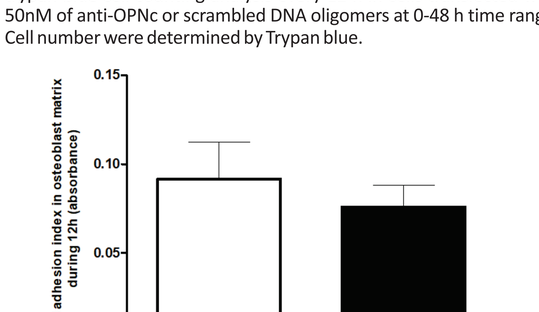
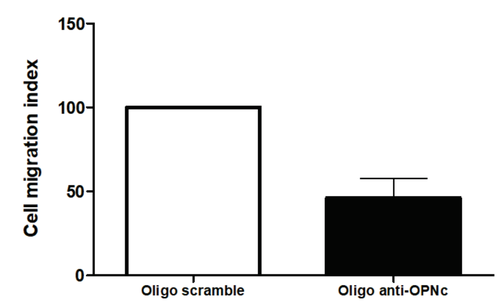


Figure 11: OPNc knock-down inhibit RS4;11 B-ALL cell adhesion rates over osteoblastic cell matrix. Bar graphs represent cell adhesion rates over extracelular matrix produced by MC3T3-E1 cell line. Cells were transfected with 50nM of anti-OPNc or scrambled DNA oligomers. Cell adhesion rates were evaluated by absorbance measurements at 650nm.



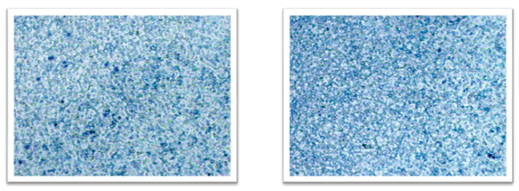
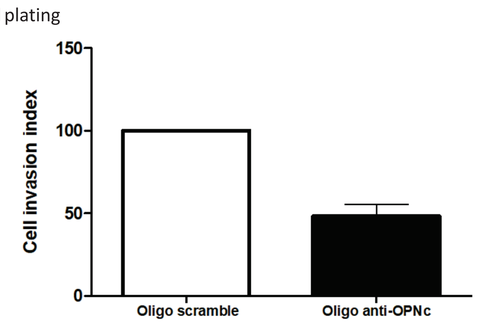


Figure 12: OPNc knock-down inhibit RS4;11 B-ALL cell migration rates. Bar graphs represent cell migration rates in boyden chamber assays. Cells were transfected with 50nM of anti-OPNc or scrambled DNA oligomers. Cell migration rates were evaluate in boyden chamber after 4h of cell plating



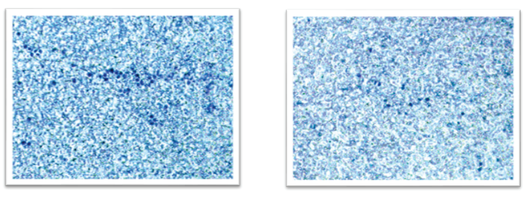


Figure 13: OPNc knock-down inhibit RS4;11 B-ALL cell invasion rates. Bar graphs represent cell invasion rates in boyden chamber coated with matrigel. Cells were electroporated with 50nM of DNA oligomers specifically targeting OPNc (anti-OPNc) ou scrambled DNA oligomers . Cell invasion rates were evaluate in boyden chamber coated with matrigel at 24h time point.

CONCLUSIONS

In summary, these data provide evidence that OPNc splice variant may control the leukemic cell's ability to detach from the bone marrow matrix and could also be a negative modulator of cell proliferation, while inducing cell invasion properties. These features could then favor central nervous system infiltration, contributing to a more aggressive B-ALL phenotype.

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