Osteopontin-c is overexpressed and mediates cell adhesion and proliferation in leukemia cell line with *KMT2A-AFF1*

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for 25% of all childhood cancers. Detection of specific gene rearrangements allows the identification of relevant prognostic subgroups of childhood B-cell precursor ALL (BCP-ALL). Among the gene products of altered expression in BCP-ALL is osteopontin (OPN), a matricelullar protein known to be an important solid tumor biomarker. OPN transcript suffers alternative splicing generating at least three OPN splicing isoforms (OPN-SI), however its roles in hematological malignancies are still under investigation.

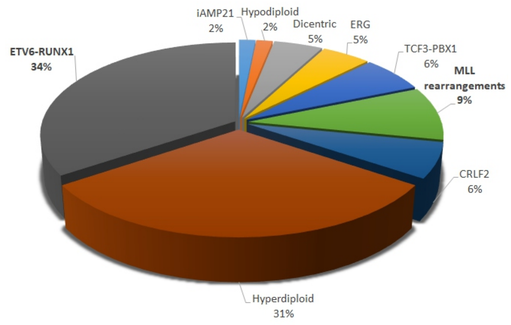


Figure 1: Frequency of molecular-cytogenetic abnormalities in B-ALL. Rearrangements of the *KMT2A* (also known as mixed lineage leukemia (MLL) gene located on chromosome 11q23 are observed in more than 80% of infant ALL and are related to poor prognosis. The translocation t(12;21) (p13;q22) results in the *ETV6-RUNX1* fusion gene, which is the most common rearrangement associated with a good prognosis in ALL. Adapted from Mullighan et al., 2012

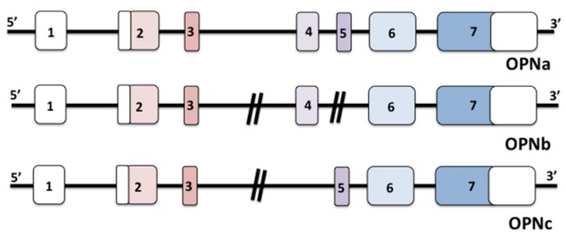


Figure 2: OPN gene structure, described splice variants and their respective exon arrangements. The full length isoform, named OPNa, contains 7 exons represented by white (non-coding) and colored (coding) boxes. While OPNb and OPNc lack exons 5 and 4, respectively (Bastos et al., 2017).

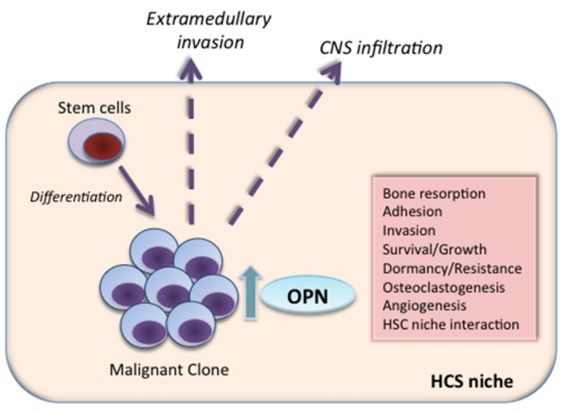
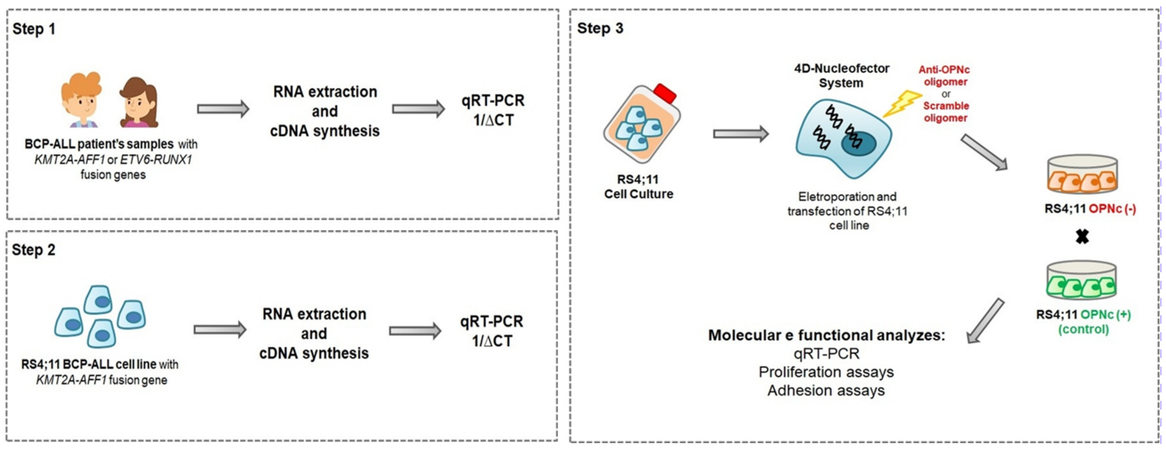


Figure 3: OPN perform distinct roles in HMs. In the bone marrow (BM) niche, upregulated OPN secreted by malignant clones, as well as by additional cells present in the BM microenvironment (e.g. osteoblasts) can promote differentiation of hematopoietic progenitors and stem cells. Overexpressed OPN can 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).

OBJECTIVES

In order to investigate the expression patterns and putative roles of OPN splice variants in childhood B-cell precursor acute lymphoblastic leukemia, this study aims to analyze the expression of OPN-SIs in BCP-ALL samples and then investigate the role of the most overexpressed OPN-SI in these patients on modulating cellular and molecular aspects of BCP-ALL with *KMT2A-AFF1*.

METHODOLOGY



CONCLUSION

- The three tested OPN-SI are expressed in most tested BCP-ALL patient samples, including both favorable and unfavorable prognostic subgroups represented by typical gene rearrangements;
- Our data provide early evidence that these OPN-Sis could differently contribute to distinct types of BCP-ALL leukemia;
- OPNc expression levels is associated with some worse prognostic features, such as CNS infiltration and WBC counting, as well as, NCI risk stratification; Our data suggest that OPNc modulates cell adhesion and proliferation rates of RS4;11 leukemia cell line exhibiting *KMT2A-AFF1* fusion gene, collaborating for maintenance of leukemic phenotype;
- Based on these OPN-SIs expression patterns, further work should be conducted in order to investigate their putative applications, specially for OPNc, as additional risk-stratification and prognostic markers for BCP-ALL.

RESULTS

Table 1. Clinical features about BCP-ALL patients

Clinical Features	All Patients (N(%))	*KMT2A-AFF1* positive (N(%))	*ETV6-RUNX1* positive (N(%))
Age groups (years)			
< 1 year	19 (35.18)	18 (69.23)	1 (3.57)
1. – 10 years	4 (7.40)	7 (26.92)	24 (85.71)
>10 years	31 (57.40)	1 (3.84)	3 (10.71)
Sex			
Female	29 (53.70)	12 (46.15)	17 (60.71)
Male	25 (46.29)	14 (53.84)	11 (39.28)
Initial WBC			
< 50 x 10³ céls./mm³	28 (51.85)	8 (30.76)	20 (71.42)
≥ 50 x 10³ céls./mm³	26 (48.14)	18 (69.23)	8 (28.57)
NCI Risk Group			
Standard risk (SR)	21 (38.88)	1 (3.84)	20 (71.42)
High risk (HR)	33 (61.11)	25 (96.15)	8 (28.57)
CNS status			
Infiltration	10 (18.51)	9 (34.61)	1 (3.57)
No infiltration	44 (81.48)	17 (65.38)	27 (96.42)
Total	N = 54	N = 26	N = 28

Abbreviations: CNS, central nervous system; NCI, National Cancer Institute; WBC, white blood cell

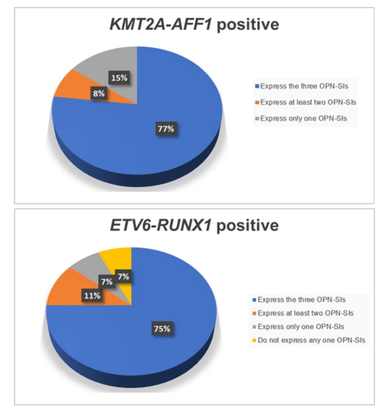


Figure 4: Frequency of OPN-SIs expression levels in BCP-ALL patient samples. The frequency of OPN-SIs expression levels in BCP-ALL patients subgroups with *KMT2A-AFF1* fusion gene or ETV6-RUNX1 rearrangement, as a comparative group.

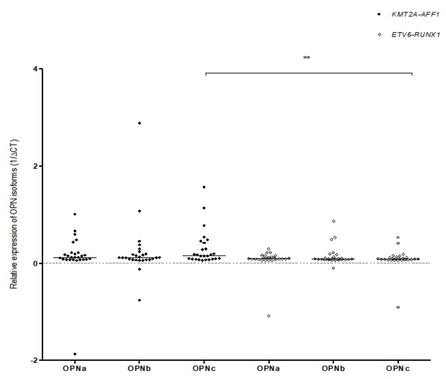


Figure 5: Expression levels of OPN-SIs in BCP-ALL patient samples. The mRNA expression levels of OPN-SIs in BCP- ALL patient samples were analyzed using real-time RT- PCR. Dot-plot graph represent OPN-SI relative expression levels as demonstrated by 1/Delta CT. Actin gene has been used as the reference gene. In patient samples harbouring either *ETV6-RUNX1* or *KMT2A-AFF1* rearrangements, OPNa and OPNc isoforms are more expressed, respectively. Additionally, patients harbouring *KMT2A- AFF1* fusion exhibit higher OPNc transcriptional levels those harbouring ETV6-RUNX1 rearrangement (p=0.0056).

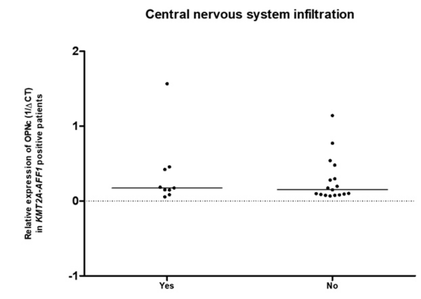


Figure 6: OPNc transcriptional levels and association with central nervous system (CNS) infiltration in patients with *KMT2A-AFF1* fusion gene. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients *KMT2A-AFF1* positive with CNS infiltration present higher median OPNc transcriptional expression levels than those patient samples without CNS infiltration.

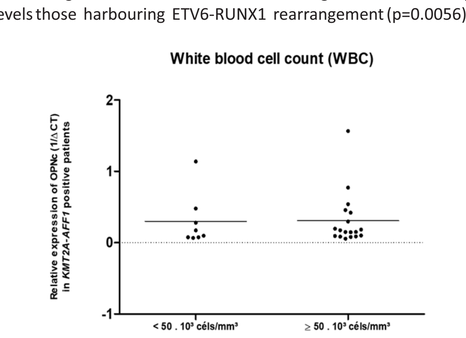


Figure 7: OPNc transcriptional levels and association with initial WBC counting in patients with *KMT2A-AFF1*. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients KMT2A-AFF1 positive with initial WBC counting greater than 50,000 céls./mm³ present higher OPNc transcriptional expression levels than patients with WBC less than 50,000 céls./mm³

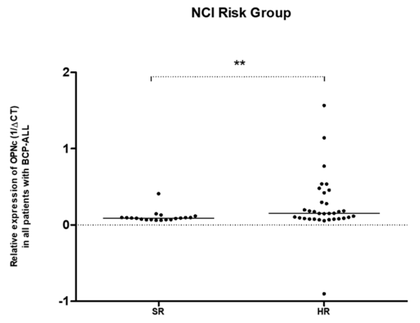


Figure 8: OPNc transcriptional levels and association with National Cancer Institute (NCI) risk-based therapy group stratification. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients classified in high risk (HR) of relapse group present higher OPNc transcriptional expression levels than patients classified standard risk (SR) of relapse group (*p<0,05).

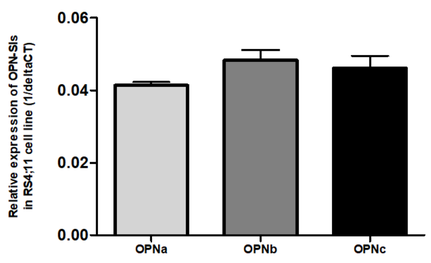


Figure 9: Relative expression of OPN-SIs in RS4;11 CPB cell line with *KMT2A-AFF1* fusion gene. The mRNA expression levels of OPN-SIs in RS4;11 cell line were analyzed using real-time RT-PCR. Column bar graph represent OPNa, OPNb and OPNc relative expression levels as demonstrated by 1/Delta CT, respectively. Actin gene has been used as the reference gene.

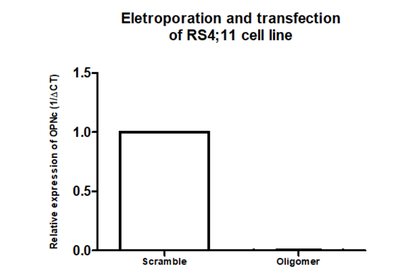


Figure 10: Efficiency of RS4;11 CPB-ALL cell line electroporation and transfection by 4D-Nucleofector system with 50 nM oligomers scramble and anti-OPNc after 36h.

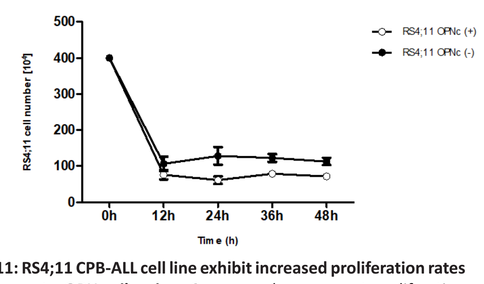


Figure 11: RS4;11 CPB-ALL cell line exhibit increased proliferation rates in response to OPNc silencing. Curve graphs represent proliferation rates according to trypan Blue cell counting analysis every 12 hours in cells electroporated with 50nM of DNA oligomers specifically targeting OPNc (anti-OPNc) ou scramble DNA oligomers. Cell number were determined by Trypan Blue assay every 12 hours.

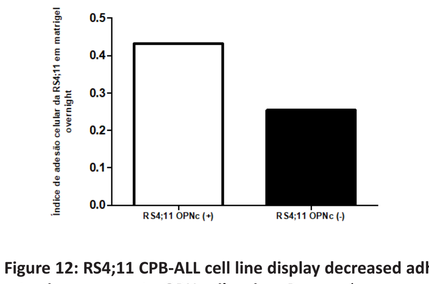


Figure 12: RS4;11 CPB-ALL cell line display decreased adhesion rates in response to OPNc silencing. Bar graphs represent cell adhesion rates in matrigel. Cells were electroporated with 50nM of DNA oligomers specifically targeting OPNc (anti-OPNc) ou scrambled DNA oligomers . Cell adhesion rates were evaluate **by absorbance measured at 650nm.**

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