EXPRESSION OF OSTEOPONTIN SPLICING ISOFORMS IN CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

Osteopontin (OPN) primary transcript suffers alternative splicing generating three OPN splicing isoforms (OPN-SI), named OPNa, OPNb and OPNc. In solid tumors, total OPN (tOPN) expression has been correlated to many hallmarks of cancer. However, the OPN roles in hematological malignancies (HMs) are still under investigation. Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Detection of specific gene rearrangements allows the identification of relevant prognostic subgroups of childhood B-cell precursor ALL (BCP-ALL). Patients harbouring the ETV6-RUNX1 rearrangement are classified as a good prognostic subgroup, while those with the KMT2A-AFF1 fusion are included into poor prognostic subgroup. In BCP-ALL, it has been reported that tOPN support tumor dormancy and cell survival in response to chemotherapeutic drugs. However, the specific roles of OPN-SI have not been addressed. In order to investigate the expression patterns and putative roles of these OPN splice variants in BCP-ALL, this work aimed to analyze the expression of OPN-SI in BCP-ALL samples. Key words: Osteopontin, BCP-ALL, splicing variants

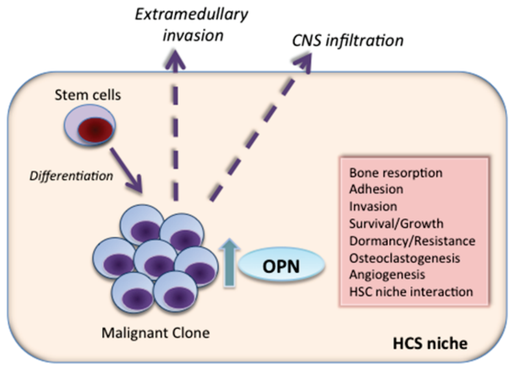


Figure 1: OPN perform distinct roles in HMs. In the bone marrow (BM) niche, upregulated OPN secreted by malignant clones, as well as by additional cells present in the BM microenvironment (e.g. osteoblasts) can promote differentiation of hematopoietic progenitors and stem cells. Overexpressed OPN can 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).

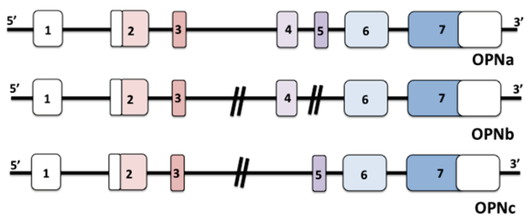


Figure 2: OPN gene structure, described splice variants and their respective exon arrangements. The full length isoform, named OPNa, contains 7 exons represented by white (non-coding) and colored (coding) boxes. While OPNb and OPNc lack exons 5 and 4, respectively.

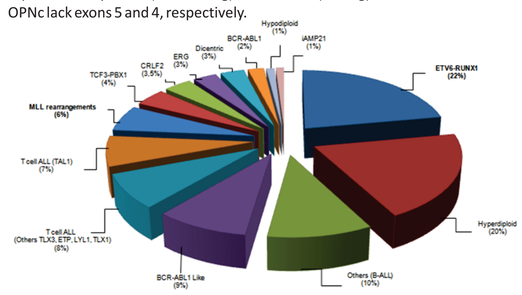
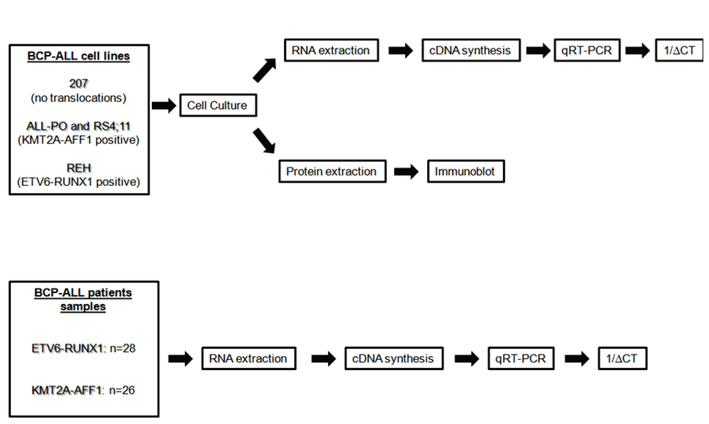


Figure 3: Frequency of molecular-cytogenetic abnormalities in ALL. Rearrangements of the KMT2A (also known as mixed lineage leukemia (MLL) gene located on chromosome 11q23 are observed in more than 80% of infant ALL and are related to poor prognosis. The translocation t(12;21) (p13;q22) results in the ETV6-RUNX1 fusion gene, which is the most common rearrangement associated with a good prognosis in ALL. Adapted from Gowda et al., 2015

METHODOLOGY



RESULTS

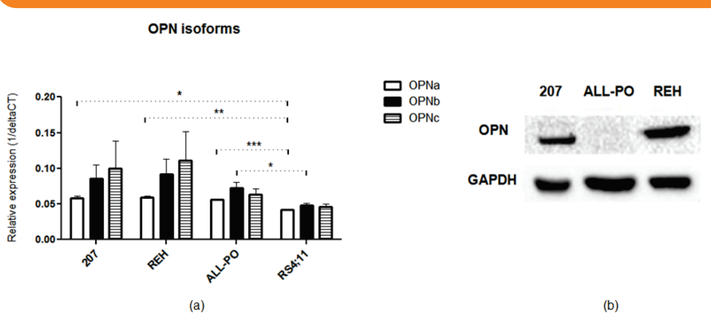


Figure 4: Expression levels of OPN-Sis in BCP-ALL cell lines. (a) The mRNA expression levels of OPN-SIs in BCP-ALL cell lines were analyzed using real-time RT-PCR. Bar graphs represent relative expression levels as demonstrated by 1/Delta CT. Actin gene has been used as the reference gene. Both 207 and REH cell lines displayed higher OPNc and OPNb levels than OPNa. Moreover, ALL-PO and RS4;11 presented similar levels of these three OPN-SIs. (b) The protein expression levels of total OPN were also analyzed by immunoblot assays using the anti-OPN O-17 polyclonal antibody. Higher tOPN protein levels have been observed in 207 and REH cell lines, while in ALL-PO protein expression has not been detected.

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Table 1. Clinical features about ALL patients

Clinical Features	*All Patients (N(%))*	*ETV6-RUNX1 positive (N(%))*	*KMT2A-AFF1 positive (N(%))*
Age groups (years)			
< 1 year	19 (35.18)	1 (3.57)	18 (69.23)
1 – 10 years	4 (7.40)	24 (85.71)	7 (26.92)
> 10 years	31 (57.40)	3 (10.71)	1 (3.84)
Sex			
Male	29 (53.70)	17 (60.71)	12 (46.16)
Female	25 (46.29)	11 (39.28)	14 (53.84)
Initial WBC			
< 50.000/µl	28 (51.85)	20 (71.42)	8 (30.76)
≥ 50.000/µl	26 (48.14)	8 (28.57)	18 (69.23)
NCI Risk Group			
Standard Risk (SR)	21 (38.88)	20 (71.42)	1 (3.84)
High Risk (HR)	33 (61.11)	8 (28,57)	25 (96.15)
CNS status			
Infiltration	10 (18.51)	1 (3.57)	9 (34.61)
No infiltration	44 (81.48)	27 (96.42)	17 (65.38)
Total	*N=54*	*N=28*	*N=26*

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

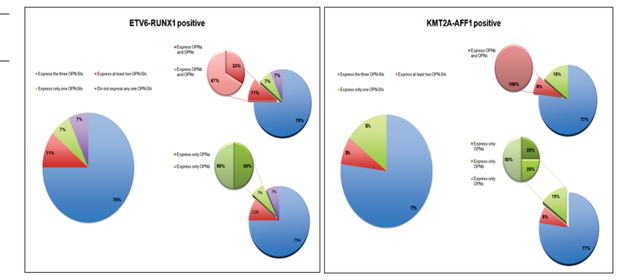

Figure 5: Frequency of BCP-ALL patients expressing OPN-SIs. (a,b) Most patients harbouring either ETV6-RUNX1 or KMT2A-AFF1 fusion express the three OPN-SIs. Only in ETV6-RUNX1 patients group some samples do not express any OPN-SIs.

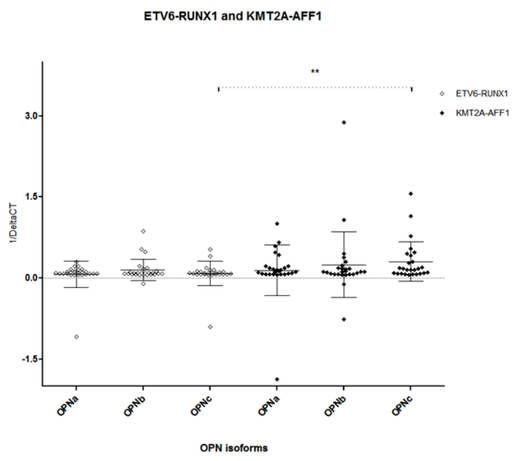

Figure 6: Expression levels of OPN-SIs in BCP-ALL patient samples. The mRNA expression levels of OPN-SIs in BCP-ALL patient samples were analyzed using real-time RT-PCR. Dot-plot graph represent OPN-SI relative expression levels as demonstrated by 1/Delta CT. Actin gene has been used as the reference gene. In patient samples harbouring either ETV6-RUNX1 or KMT2A-AFF1 rearrangements, OPNc isoforms is expressed in higher levels than OPNb and OPNa. Additionally, patients harbouring KMT2A-AFF1 fusion exhibit higher OPNc transcriptional levels those harbouring ETV6-RUNX1 rearrangement (p=0.0056).

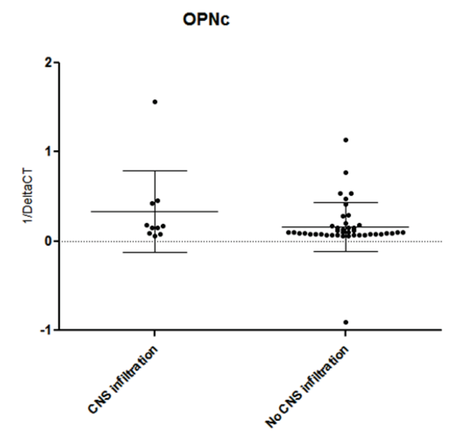

Figure 7: OPNc transcriptional levels and association with central nervous system (CNS) infiltration. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients with CNS infiltration present higher median OPNc transcriptional expression levels than those patient samples without CNS infiltration (p>0,05).

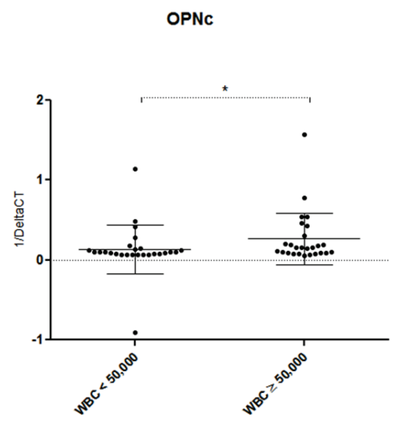

Figure 8: OPNc transcriptional levels and association with initial WBC counting. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients with initial WBC counting greater than 50,000/µl present higher OPNc transcriptional expression levels than patients with WBC less than 50,000/µl (*p< 0.05).

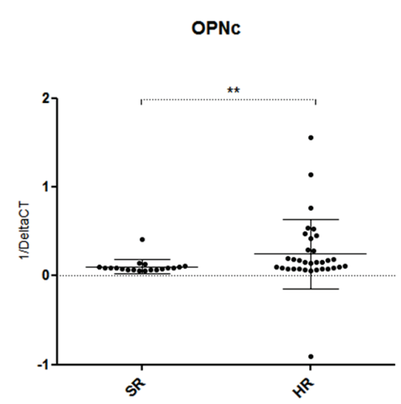

Figure 9: OPNc transcriptional levels and association with National Cancer Institute (NCI) risk-based therapy group stratification. Dot-plot graph represent OPNc relative expression levels as demonstrated by 1/Delta CT. Patients classified in high risk (HR) of relapse group present higher OPNc transcriptional expression levels than patients classified standard risk (SR) of relapse group (*p<0,05).

CONCLUSIONS

- The three tested OPN-SI are expressed in all tested BCP-ALL cell lines and most tested BCP-ALL patient samples, including both good and poor prognosis groups represented by typical gene rearrangements.
- Our data provide early evidence that these OPN-SIs could specifically contribute to distinct types of BCP-ALL leukemia
- OPNc expression levels is associated with some prognostic features, such as CNS infiltration and WBC counting, as well as, NCI risk stratification.
- Based on these OPN-SIs expression patterns, further work should be conducted to investigate their putative applications, specially for OPNc, as additional risk-stratification and prognostic markers for BCP-ALL, as well as, their roles on modulating HM progression.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA