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Oncogenic Transcription Factors in T-cell Acute Lymphoblastic Leukemia: a review and identification of a novel player – CASZ1

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“Doctors are men who prescribe medicine of which they know little, to cure diseases of which they know less, for human beings of which they know nothing.”

Voltaire

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) corresponds to a lineage of the commonest childhood cancer – ALL. Childhood T-ALL 5-year overall survival is around 80%, still 10% behind B-ALL's. This is explained by the fact that B-ALL has a classification with well-defined prognostic and therapeutic implications; on the contrary, T-ALL still lacks a comprehensive genomic classification, which ultimately lead to increased chemoresistance and worse survival.

The objective of the current work is to systematically review the literature in what regards the known T-ALL subgroups, which are based on the enrichment for particular oncogenic transcription factors.

These T-ALL subgroups are based on deregulated transcription factor (TF) genes, a hallmark of T-ALL, with these being - TAL1, TAL2, TLX1, TLX3, HOXA, LMO1/LMO2, LMO2/LYL1 and NKX2-1. By reviewing the different genetic background of each subgroup, new therapeutics can be tailored to specific genetic abnormalities that characterize a determined groups – e.g. inhibition of PI3k pathway in TAL1+; inhibition of JAK-STAT pathway in HOXA+; and inhibition of the fusion protein NUP214-ABL1 in TLX1+, for instance.

In addition, the host laboratory identified a new transcription factor with impact on T-ALL (CASZ1), which is a TAL1 target and also behaves as an oncogene. CASZ1 has been shown to mediate its oncogenic action by increasing cell growth and viability through the PI3K pathway. My contribution to this work is also included in the present work.

The literature review and scientific experiments reported herein are aligned in their focus on transcription factors with relevance for the biology of T-ALL and in the evidence proposing the tackling of PI3K pathway in TAL1+ T-ALL cases.

Keywords

T-cell acute lymphoblastic leukemia; Transcription Factors; Oncogenic Pathways; TAL1; CASZ1

Resumo

A Leucemia Linfoblástica Aguda de células-T (LLA-T) corresponde a uma linhagem do mais comum cancro da infância – LLA. A sobrevivência das crianças com LLA-T ronda os 80%, ainda 10% atrás da LLA-B. Isto é explicado pelo facto de que a LLA-B tem uma classificação com implicações prognósticas e terapêuticas bem definidas; pelo contrário, a LLA-T, carece de uma classificação genómica com validade prognóstica que leva a uma maior quimiorresistência e pior sobrevivência dos doentes.

O objetivo do presente trabalho é rever sistematicamente a literatura no que toca aos vários subgrupos de LLA-T baseada em fatores de transcrição (FT) oncogénicos específicos.

Estes subgrupos são baseados na desregulação de FT cruciais na LLA-T, sendo estes - TAL1, TAL2, TLX1, TLX3, HOXA, LMO1/LMO2, LMO2/LYL1 e NKX2-1. Ao rever-se o diferente panorama genético de cada um destes subgrupos, novas terapêuticas poderão ser desenhadas por forma a reverter as anomalias genéticas que os caraterizam, como por exemplo – inibição da via PI3k em TAL1+; inibição de JAK-STAT em HOXA+; e inibição da proteína de fusão NUP214-ABL em TLX1+.

O laboratório, onde o trabalho experimental foi desenvolvido, identificou um novo FT (CASZ1) alvo de TAL1 que também se comporta como um oncogene. Este gene provou mediar a ação oncogénica de TAL1 ao aumentar a viabilidade e crescimento celular através da via PI3K. O meu contributo para este trabalho está aqui incluído.

Deste modo, a revisão e trabalho experimental estão alinhados no foco dado aos fatores de transcrição como sendo relevantes para a biologia de LLA-T e na evidência que propõe a inibição da via PI3K em T-ALL do subgrupo TAL1+.

Palavras-chave

Leucemia Linfoblástica Aguda de células-T; Fatores de Transcrição; Vias oncogénicas; TAL1; CASZ1.

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REVIEW

Search Method: In August and September 2021, we searched on PubMed for reviews with the following MeSH Terms – "Precursor cell lymphoblastic leukemia lymphoma" and "transcription factors" published between 2015 and 2021. From those 95 obtained results we analysed a group of reviews that focused mainly on T-ALL. When a mutation or experiment were mentioned on those reviews, we went back to the original research article that initially conducted the described experiment.

ALL

Acute lymphoblastic leukemia (ALL) is the commonest childhood cancer (Roberts & Mullighan, 2015). ALL is a malignant clonal disease that develops when a lymphoid progenitor cell becomes genetically altered through somatic changes and undergoes differentiation arrest and uncontrolled proliferation. These malignant cells will then proliferate in the bone marrow, blood and extramedullary sites.

Epidemiology

ALL has an estimated incidence of 1.6 per 100 000 population and 80% of its cases happen in children (National Cancer Institute, 1975-2013). Two different lineages of ALL are described: T-cell (T-ALL) and B-cell (B-ALL). T-ALL represents 10–15% of paediatric and 20–25% of adult cases of ALL in Europe, the United States and Japan, being more prevalent in males than in females (72% VS 28%) (DeAngelo, 2005).

Clinical features

Most of the clinical manifestations of ALL reflect the accumulation of lymphoblasts within the bone marrow, peripheral blood and extramedullary sites. Marrow failure signs include anemia, thrombocytopenia and leukopenia. B-symptoms (fever, weight loss, night sweats) are part of the unspecific presentation of ALL leading to the need of a broad differential diagnosis. Extramedullary site infiltration manifests as – lymphadenopathy, hepatosplenomegaly, meningismus (due to CNS infiltration) and mediastinal mass. Moreover, children typically appear with bone pain (Terwilliger & Abdul-Hay, 2017).

Diagnosis

Diagnosis is established by the presence of 20% or more lymphoblasts in the bone marrow or peripheral blood. Bone marrow aspirate and/or biopsy are sent for morphology, flow cytometry (T-cells are CD3+ and negative for B-cell markers and MPO) and cytogenetic testing to both confirm the diagnosis and classify patients in different risk categories. Lumbar puncture with cerebrospinal fluid (CSF) analysis is standard of care at the time of diagnosis to evaluate for CNS involvement, which occurs in 5-8% of patients. Laboratory evaluation (LE) includes differential blood count and peripheral blood smear to evaluate the other hematopoietic cell lineages, coagulation profiles and serum values such as uric acid, calcium, phosphate and lactate dehydrogenase to evaluate a possible tumour lysis syndrome. (Alvarnas et al., 2015).

Treatment

ALL treatment consists in chemotherapy schemes in 3 phases – induction, consolidation and long-term maintenance. Induction includes vincristine, corticosteroids and an anthracycline. L-asparaginase and cyclophosphamide may be added depending on the protocol that is followed by the cancer centre. Intratechal methotrexate and cranial radiation are added if CNS infiltration is positive. After induction some high-risk (age > 35 or white blood cells $>100*10^9$) patients with a matched-donor may undergo Allo-SCT (Stem Cell Transplant). If not, a consolidation and maintenance regimes may be started, the latter usually lasts 2-3 years. (Terwilliger & Abdul-Hay, 2017).

Childhood B-cell ALL (B-ALL) has a 5-year patient survival over 90-95%, in opposition T-ALL's 5-year survival rate is still behind at 80-85% (Teachey & Pui, 2019b). In adults, the ALL (B and T-lineage) survival rates stagnated in 55% (Todeschini et al., 1998). B-ALL classification has reached a consensus with prognostic and therapeutic implications since genetic alterations can be combined with clinical variables to risk stratify patients into prognostic groups, while T-ALL still lacks a comprehensive classification, because none of its common alterations have been shown to predict outcome independently (Teachey & Hunger, 2013). Only Minimal Residual Disease (MRD) of $> 10^{-4}$ (fraction of leukemic cells by PCR) after induction/consolidation defines a clear unfavourable prognosis (Teachey & Pui, 2019a). T-ALL patients are usually older, more frequently

black (two populations associated with worse outcomes) and have a higher probability of relapse, CNS infiltration and chemotherapy resistance (Pieters et al., 2016) (Belver & Ferrando, 2016).

T-ALL

To sum-up, T-ALL, also called precursor T-cell acute lymphoblastic leukemia, is a disease that arises from T lymphoblasts at varying stages of differentiation. Leukemia and Lymphoma overlap in terms of clinical presentation, however, the term lymphoma is commonly applied when there less than 20% of bone marrow involvement in conjunction with the detection of a mass lesion.

Once the diagnosis is suspected, the clinical doctor must proceed with the diagnostic procedures explained previously, while being reminded that most patients initially require transfusion support, treatment of infections and correction of metabolic imbalances, such as hyperuricemia.

Despite the relatively good survival with the existing therapies, two challenges remain: death from toxicity, but also to improve the rationality that is still lacking, and the risk stratification compared to B-ALL (Table 1.).

B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with iAMP21
B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);TCF3-PBX1
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) IL3-IGH
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1);ETV6-RUNX1
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); KMT2A rearranged
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);BCR-ABL1
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma, NOS
T-lymphoblastic leukemia/lymphoma

Table 1. Adapted from the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.

The transcription factor oncogenes that help explain the genetic and survival differences in T-ALL will be reviewed here.

GENETICS

The genetic alterations are part of a multistep process which involves the creation of oncogenic pathways that alter cell growth, proliferation, survival and differentiation during thymocyte development (Campos-Sanchez et al., 2011). Therefore, T-ALL may develop in any step from the bone marrow migration to intrathymic development.

T-cell Lymphocyte Development

In the bone marrow, uncommitted haematopoietic precursors develop into either B or T cell lineages depending on the different transcription factors expressed. Notch-1, a member of the Notch family, is activated in lymphoid progenitor cells by the ligands Jagged/Delta-like family, expressed on the surface of thymic epithelial cells, and collaborates with a transcription factor called GATA-3 to commit developing lymphocytes to the T lineage (Bommhardt et al., 2004). The thymus, which is the primary site of T cell development, is where developing T cells undergo T cell receptor (TCR) rearrangement. The lymphoid double-negative (DN; CD4−CD8−) progenitors enter the thymus at the cortico-medullary junction. These double-negative thymocytes can be subdivided into four stages of differentiation based on the expression of CD44 and CD25: DN1, CD44+CD25−; DN2, CD44+ CD25+; DN3, CD44−CD25+; and DN4, CD44− CD25−. As cells differentiate into DN3 cells they express the pre-T cell receptor (pre-TCR), which is composed of an invariable pre-TCRα chain and a rearranged TCRβ. Pre-TCR signalling induces marked proliferation of DN4 cells and promotes their maturation to the CD4+CD8+ double-positive (DP) stage of differentiation. As T cells stop proliferating, they rearrange their *TCRA* gene (encoding TCRα). The expression of a complete TCR composed of TCRβ and TCRα chains enables the interaction of DP thymocytes with major histocompatibility complex (MHC) expressed on the surface of thymic epithelial cells. Active recognition of MHC by the TCR results in a survival signal (positive selection) and commitment of the T cell towards the CD8+ or the CD4+ lineage, depending on whether it recognizes MHC class I or MHC class II, respectively. However, a strong

interaction between the TCR and MHCs loaded with self-peptides in the medulla induces apoptosis (negative selection). Single-positive CD4+and CD8+ cells that survive negative selection leave the thymus and populate the peripheral lymphoid organs (Abbas, 2007) (Belver & Ferrando, 2016).

Immunophenotype

Thymocyte development spectrum has been associated with various gene expression profiling categories. Across this spectrum, three main T-ALL immunophenotypes have been described: early T-cell precursor (CD4-CD8-), early cortical stage thymocyte (CD4+CD8+CD1a+) and late cortical stage thymocyte (CD4+CD8+CD3+). (A. A. Ferrando et al., 2002). Early T-cell precursor (ETP)-ALL are close related to hematopoietic stem cells and myeloid progenitors, and their pattern of mutations overlaps with acute myeloid leukemias (AML). Since ETP-ALL has such a different genetic background, another immunophenotypic classification could be seen - ETP-ALL, near-ETP-ALL and non-ETP-ALL (Y. Liu et al., 2017).

Through Genome-wide Analysis (transcriptome, whole exome sequencing, single-nucleotide polymorphism microarray), eight genetic subgroups based on the deregulation of nine different transcription factor oncogenes have been defined - TAL1, TAL2, TLX1, TLX3, HOXA, LMO1/LMO2, LMO2/LYL1 and NKX2-1. These recent genetic patterns are closely related to the previously stated immunophenotypic classification. ETP-ALL is characterized by LMO2/LYL1 deregulation. Early cortical thymocyte leukemias are primarly associated with translocations resulting in aberrant expression of TLX1, TLX3, NKX2-1 and HOXA. Late cortical leukemias usually overexpress TAL1 with either LMO1 or LMO2 (A. Ferrando, 2018) (Y. Liu et al., 2017).

Subtype	Total	ETP		Near-ETP		Non ETP	
		N	%	N	%	N	%
HOXA	27	3	15,80%	2	8,30%	22	15,10%
LMO1	1	0	0,00%	0	0,00%	1	0,70%
LMO2	7	1	5,30%	0	0,00%	6	4,10%
LMO2/LYL1	13	7	36,80%	2	8,30%	4	2,70%
NKX2-1	11	0	0,00%	0	0,00%	11	7,50%
TAL1	52	2	10,50%	10	41,70%	40	27,40%
TAL2	7	0	0,00%	1	4,20%	6	4,10%
TLX1	19	1	5,30%	0	0,00%	18	12,30%
TLX3	33	4	21,10%	7	29,20%	22	15,10%
NA	19	1	5,30%	2	8,30%	16	11,00%
Total of patients	189	19		24		146	

Table 2. Adapted from the article *The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia (Y. Liu et al., 2017)*

Despite these efforts, the immunophenotype and genetic classification still lack prognostic or treatment response predictive value. Yet, these recent genetic insights will be of crucial importance to develop targeted therapies (Lato et al., 2021). In contrast to B-ALL, where certain genetic abnormalities (e.g. ETV6-RUNX1 and high hyperdiploidy – 50% B-ALL patients) are clearly associated with good treatment prognosis (90% 5-year overall survival), in T-ALL the prognosis is uncertain (Pieters et al., 2016). Only ETP-ALL has been described as having poor prognosis (Richard-Carpentier et al., 2019).

T-ALL genetic landscape is marked by the transcriptional activation of several protooncogenes, submicroscopic deletions of cancer suppressor genes, epigenetic deregulation, ribosomal dysfunction, altered RNA stability, cell-cycle dysregulation, and disordered signaling pathways – e.g. NOTCH1/FBXW7, PI3K/Akt/mTOR, RAS/MAPK, and IL7R–JAK–STAT (Gianni et al., 2020). In-depth characterization of all the T-ALL landscape goes over the purpose of the current work. Therefore, we will focus on the previously described transcription factor oncogenic subgroups and how they can be targeted.

TRANSCRIPTION FACTOR-BASED CLASSIFICATION

Transcription factors (TFs) are proteins capable of binding DNA in a sequence-specific manner and regulate transcription of target genes. TFs represent around 8% of human coding genome and are responsible for processes that specify cell types, control specific pathways and drive cell differentiation. Over 1600 proteins may be classified as TFs, which are organized in different families based on the DNA-binding domain or motif (DBD) similarities (Lambert et al., 2018). DBDs are a wide range of protein structural forms that make the recognition of DNA sequences possible through the identification of longer sequences – promoters, enhancers and silencers. TFs act by recruiting cofactors involved in chromatin and nucleosome remodelling and modification of histones and other proteins. Therefore, they may act as either activators or repressors, or both, depending on the context of the transcription (Weirauch & Hughes, 2014).

Multiple families of TFs can be described, with four being important in the context of T-ALL - C2H2 zinc finger, Homeobox, bHLH (helix-loop-helix) and basic leucine zipper (bZIP) (Lambert et al., 2018). All the TFs that define the different T-ALL subgroups belong to one of these families.

Since TFs can modulate the transcription of many DNA sequences, its mutations are described as being highly deleterious, especially in cancer. In T-ALL, the nine previously described transcription factors behave as oncogenes. They can be overly expressed through multiple mechanisms –translocations (e.g. juxtaposition of T-cell receptor and oncogenes), insertions in non-coding regions and duplications. The mechanisms that drive oncogene activation and how they can be targeted will be described here. The review of the underlying mechanisms of deregulated TF expression is of crucial importance to pave the way for a rational risk-stratification in T-ALL.

Crucial TFs that do not define T-ALL subgroups

NOTCH-1 [cytogenetic band of the gene that encodes the TF (cb): 9q34.3]

NOTCH1 is a class I transmembrane glycoprotein that functions as a ligand-activated transcription factor. Its activating mutations are present in at least 60% of T-ALL cases

(Y. Liu et al., 2017). Within the hematopoietic system, NOTCH signaling plays a crucial role in T-cell lineage specification since its inhibition results in T-cell development failure and B-cell development (Xiaoyu L, 2011).

The NOTCH signaling pathway induces a transcriptional program in T-ALL responsible for the up-regulation of genes involved in anabolic cell growth and metabolism, being MYC, another key oncogenic TF, a major direct target. This pathway also regulates the expression of proteins involved in cell cycle progression, such as Cyclin D3 and cyclin-dependent kinases 4 and 6 (CDK4 and CDK6) and induces the activation of PI3K/Akt pathway through the up-regulation of HES1 and consequent transcriptional downregulation of PTEN (Oliveira et al., 2017).

Leucine-zipper

This TF family belongs to the basic superfamily, the same as bHLH family. Both have a basic α-helical DNA-contacting region. What differentiates them is the dimerization interface, this one being a leucine zipper instead of a HLH (Weirauch & Hughes, 2014).

MYB (cb: 6q23.3)

MYB oncogene encodes a leucine zipper family TF and corresponds to the second most frequently mutated oncogene, after *NOTCH1*, that is not associated with a T-ALL subgroup – 5.8% (not included in Table 2 since it does not define a subgroup) (Y. Liu et al., 2017). This suggests that MYB drives oncogenic pathways in multiple stages of T-cell maturation, thereby not being associated with a specific immunophenotype. Its over-expression may be a result of amplification, t(6;7)(q23;q32) translocation or even duplication (Gianni et al., 2020).

MYB is implicated in proliferation, survival and differentiation of hematopoietic progenitor cells, being turned-off as maturation occurs. Its overexpression is associated with a very rare form of T-ALL in children younger than 3 years-old (median of 2.2) (Clappier et al., 2007).

MYC (cb: 8q24.21)

The *MYC* oncogene encodes a leucine zipper transcription factor that functions as a keymaster regulator of cell growth and proliferation and is broadly involved in the pathogenesis of human cancer (Dang, 2012) .

MYC is a mediator of NOTCH1-induced transformation, therefore they both collaborate to activate a common transcriptional program controlling leukemia cell growth and metabolism – mainly through mTOR (*Chan*, 2007).

TFs that define T-ALL subgroups

Homeobox family – HOXA, NKX2-1, TLX1, TLX3

Homeobox (*HOX*) genes encode a group of strongly conserved TFs involved in cell lineage specification, body patterning and embryonic organogenesis. Part of the HOX genes encode a 60 aminoacid residue polypeptide, called homeodomain - the DNA-binding domain. One of the many HOX gene subclasses are HOXL and NKL. *HOXA* genes like HOXA 9, 10 and 11 belong to the former, while NKX2-1, TLX1 and TLX3 are amongst the latter subclass (Holland et al., 2007).

HOXL subclass - *HOXA* (cb – 7p15.2)

HOXA genes can be deregulated in T-ALL due to chromosomal translocations and inversions that relocate *HOXA* gene closer to *TCRB/G* loci (Soulier et al., 2005).

The HOXA mutation contributes to T-ALL in part because of its statistically significant co-occurrence with JAK3 mutations. A recent study showed that HOXA9 overexpression in co-operation with JAK3-activating mutations lead to leukemia through STAT5 increased activation thereby enhancing its transcriptional activity. This fact elucidates why HOXA+ T-ALL subgroups are enriched in JAK-STAT mutations. Targeting JAK-STAT pathway in HOXA+ T-ALL has emerged as a rational approach which proved to be effective. Dual inhibition with Ruxolitinib (JAK kinase inhibitor) and PIM1 kinase (downstream target of JAK-STAT pathway) inhibitor (AZD1208) have proven to provide strong therapeutic benefit ex and in vivo (de Bock et al., 2018).

HOXA10 and HOXA11 can be aberrantly expressed due to a chromosomal inversion [inv(7)(p15q34)] which drives their transcriptional upregulation (Speleman et al., 2005).

NKL subclass - *TLX1* (cb – 10q24.31)**, *TLX3*** (cb – 5q35.1) **and *NKX2-1*** (cb – 14q13.3)

TLX1, TLX3 and *NKX2-1* form, as all the other previous TFs oncogenes, distinct subgroups of T-ALL characterized by their over-expression. The t(10;14)(q24;q11) translocation places *TLX1* under the control of *TCRA/D* gene enhancers and results in TLX1 overexpression. On the other hand, the t(5;14)(q35;q32) translocation places TLX3 under the control of T-cell regulatory elements near the *BCL11B* locus (Gianni et al., 2020), while the *NKX2-1* inversion to the *TCRA* gene and the translocation t(7;14)(q34;q13) to the *TCRB* locus drive the overexpression of NKX2-1 (Homminga et al., 2011).

TLX1+ and TLX3+ typically display an early cortical phenotype, even though TLX3+ subgroups may also characterize some ETP-ALL phenotypes. NUP214-ABL1, a common fusion protein in T-ALL, is almost exclusively present in TLX1/3 subgroups. TLX1 and NUP214-ABL1 have been shown to cooperate in the development of aggressive leukemias (Quintás-Cardama et al., 2008). Targeting this aberrant protein with tyrosine kinase inhibitors has proved to be effective in a few T-ALL patients (Deenik et al., 2009). While, similar to *HOXA* genes, both are rich in JAK-STAT pathway mutations, they show different oncogenic pathway activation. TLX1+ is enriched in NOTCH1 mutations, while TLX3+ cases have common epigenetic mutations (Y. Liu et al., 2017). These subgroups have revealed new potential lines of treatment – NUP214-ABL1 inhibition using inhibitors of ABL1 kinase in TLX1+ and epigenetic modulators in TLX3+ – that are yet to be clinically explored.

NKX2-1 expressing cases show a TLX1-like gene expression signature and development arrest (Homminga et al., 2011). However, the two subgroups have differences. NKX2-1+ cases commonly display *LEF1* mutations, while, on the other hand TLX1+ are enriched in mutations affecting *BCL11B* and *MYC* expression (Y. Liu et al., 2017).

Zinc-finger family - LIM-domain only (LMO)

LMO are proteins that belong to the Zinc-finger family of TFs (Cassandri et al., 2017). These proteins do not bind DNA directly but contain cysteine-rich motifs in the LIM domains, which are responsible for protein-protein interactions. LIM domains behave as bridges responsible for the formation of leukemogenic transcription complexes in conjunction with bHLH family TFs (Grütz et al., 1998).

LMO1 (cb – 11p15.4) **and *LMO2*** (cb – 11p13)

LMO1/2 are activated mostly because of chromosomal translocations t(11;14)(p15;q11) and t(11;14)(p13;q11), respectively, that juxtapose the coding regions of these genes to the regulatory regions of the *TCR* loci. LMO1/2 may also be upregulated through other types of genetic alterations. LMO1 up-regulation can also be driven by a mutation at the promoter that creates a binding site for MYB. While, LMO2 can be upregulated as a result of noncoding mutations in the promotor and also because of small deletions (Gianni et al., 2020).

LMO2, as TAL1, is also a regulator of early stages of hematopoiesis at the level of pluripotent stem cell. The two genes are co-expressed in erythroid progenitors forming transcriptional complexes, thereby suggesting the synergy of these proteins (Valge-Archer et al., 1994). Likewise, LMO2 and TAL1 have been shown to also act synergistically by contributing to leukemogenesis in T-ALL through the formation of transcriptional complexes (see next) that disrupt the balance between heterodimer formed by TAL1 and an E-protein (Rabbitts, 1998).

LMO TFs that are aberrantly expressed mainly present in two distinct phenotypes – late cortical and ETP. TAL1 mutation with either LMO1 or LMO2 are characteristically associated with late cortical immunophenotype. On the other hand, ETP-ALL are enriched in both LMO2 and LYL1 mutations (Y. Liu et al., 2017). Even though TAL1 and LYL1, both bHLH TFs, drive leukemogenesis by acting synergistically with LMO2, the differentiation arrest in the thymocyte development is different (McCormack et al., 2013). This demonstrates the complex genetic landscape of T-ALL, because the same immunophenotype can have different driving mutations (late cortical – LMO and TAL1)

but also that the same aberration can be present in different immunophenotypes (LMO2 – late cortical and ETP).

bHLH family - TAL1, TAL2, LYL1

The basic Helix-loop-Helix (bHLH) family is a large superfamily of TFs. It is characterized by a HLH motif of 60 aminoacids that dimerize with other bHLH TFs forming homo or heterodimers. The bHLH may be divided in class A or I which are ubiquitously expressed, while class B or II are expressed in a tissue specific manner (Skinner et al., 2009).

TAL1 (cb – 1p33)

Last but not least, the TAL1 gene (also known as stem cell leukemia, SCL) encodes a class II bHLH transcription factor that heterodimerizes with a class I E-protein (E2A and HEB tumor suppressor genes) that binds DNA in specific sequences called E-boxes (Hsu et al., 1994). It is overexpressed in around 60% of T-ALL patients, whose immunophenotype is typically described as late cortical thymocyte, as previously explained (Belver & Ferrando, 2016). On the contrary, TAL1 transgenic mice remain in DN thymocyte stage, showing a discrepancy relative to T-ALL patients. This may suggest that mouse studies may not reflect accurately the TAL1 impact in human disease (A. A. Ferrando et al., 2002) (O'Neil et al., 2001).

TAL1 is widely characterized as a critical regulator of hematopoiesis since it is normally expressed in hematopoietic stem cells, while its expression is repressed once cells commit to B or T-cell lineages during development (Mouthon et al., 1993). Since it is responsible for the expression of genetic programs crucial for hematopoietic cell differentiation, TAL1 is regarded as a "master" TF. TAL1 being a "master" TF is mostly explained because of its association with super-enhancers (SE), clusters of large, potent enhancer regions marked by Histone H3 lysine 27 acetylation (H3K27ac) (Whyte et al., 2013).

Leukemogenesis may be induced by TAL1 through various chromosomal rearrangements. In 11% of cases a translocation occurs juxtaposing the promoter region of TCR to the TAL1 locus (Begley et al., 1989). In 30% of cases a deletion places the TAL1

coding region under the influence of the promoter of the upstream gene SIL, generating the fusion SIL-TAL1 (Aplan et al., 1990). A heterozygous somatic mutation has been recently described which introduces binding motifs for the MYB transcription factor in a precise noncoding site. MYB binds to this site and recruits H3K27ac and up-regulates leukemogenic TFs, like TAL1 (Mansour et al., 2014).

TAL1 behaves as a transcriptional complex by forming oncogenic core regulatory circuits (CRCs). Through ChIP-seq studies, TAL1 has been shown to interact with other transcription factors in a wide downstream regulatory transcriptional network. RUNX1, GATA3 and LMO2 are among the transcription factors that form a CRC. These CRCs are regulatory loops that reinforce and stabilize gene expression in leukemic cells (Saint-André et al., 2016) and the genes involved in the CRCs, like GATA3 and LMO2, form the TAL1 complex. Therefore, TAL1 forms a multimeric complex with other TFs leading to an oncogenic reprograming that reinforces itself through CRCs (Sanda & Leong, 2017).

In contrast to what happens in normal T-cell development (TAL1 downregulation), TAL1 is upregulated by previously described mechanisms in T-ALL. E-protein dimers (codified by the *E2A* tumor suppressor gene) are dominant in normal T-cells, however, this ectopic expression of TAL1 changes the heterodimer balance which will eventually lead to TAL1 complex formation. Therefore, *E2A* genes will be less available to transcribe their target genes and T-cell differentiation will be arrested (Grütz et al., 1998) (Correia et al., 2016).

The disruption of the CRC induced by TAL1 is one way of blocking leukemogenesis, and therefore, a potential therapeutic target for T-ALL. THZ1, a CDK7 inhibitor, has been shown to have a potent antiproliferative activity on T-ALL by affecting the transcriptional program of RUNX1. RUNX1 is a downstream target of TAL1 involved in the CRC with a prominent role in TAL1+ leukemic cells. (Kwiatkowski et al., 2014).

Another way of tackling TAL1-induced leukemogenesis is by inhibiting signalling pathways commonly activated in TAL1+ T-ALL cases. TAL1+ T-ALL are enriched in PI3K pathway mutations, such as in *PTEN* and *PIK3R1*. Loss of PTEN results in constitutive activation of AKT-mTOR signalling axis, promoting cell cycle progression. Despite the multiple studies with PI3K inhibitors showing anti-leukemic activity, no study has approached this question in a TAL1+ T-ALL subgroup directly (Paganelli et al., 2019).

TAL1-related genes – *TAL2* (cb – 9q31.2) **and *LYL1*** (cb – 19p13.13)

TAL1, TAL2 and LYL1 have highly homologous bHLH domains and therefore have a common mechanism regarding malignant potential. TAL2 and LYL1 are over-expressed in T-ALL due to t(7;9)(q34;q32) and t(7;19)(q34;p13) translocations, respectively, that juxtapose the oncogenes with the transcriptional element of T-cell receptor beta-chain. (Xia et al., 1991) (Mellentin et al. 1989).

Overexpression of TAL-related oncogenes synergistically cooperates with LIM-only domain TFs (described above) through the formation of transcriptional complexes, which drive leukemogenesis (Grütz et al., 1998).

NEXT STEPS – Unraveling CASZ1, a novel TF in T-ALL

This review has briefly elucidated the importance of genomic analysis to identify the mechanisms that drive malignant transformation in different T-ALL subgroups. Examples like inhibiting RUNX1 and PI3K pathways in TAL1+, JAK3-STAT5 pathway in HOXA+ and NUP214-ABL1 in TLX1/3+ offer us new opportunities for a more rational design of tailored therapies for this still not so well characterized disease. It has been proved that there is still much work to be done in order to better stratify risk and improve patients overall survival accordingly. The next step would be to design genetic models that mirror the different T-ALL subgroups so that new therapies may be tested in mouse models and in vitro with the ultimate goal of creating clinical trials for license approval.

In accordance with the next proposed step in T-ALL genomic landscape study, preliminary data from the host laboratory identified a new TAL1 transcriptional target, a zinc-finger TF called CASZ1. This gene has two splicing forms (Zhihui Liu et al., 2006) – CASZ1a and CASZ1b and has been documented to have a role in other types of cancer either behaving as a tumor suppressor (neuroblastoma and hepatocellular cancer) or as an oncogene (ovarian cancer) (Z. Liu et al., 2011; C. Wang et al., 2012; J. L. Wang et al., 2018). Preliminary laboratory data suggest that only CASZ1b may have an impact on T-ALL progression, since it is over-expressed in T-ALL patient samples when compared to control healthy thymocytes.

SCIENTIFIC EXPERIMENT

Bearing in mind the previous review and CASZ1's relation to the major T-ALL oncogene TAL1, it is of crucial importance to unravel its functional role in T-ALL cells and ultimately discover ways to target CASZ1 potential oncogenic role in this malignancy. Thus, the main purposes of this work were to study the impact of CASZ1 overexpression and to discover how to tackle its oncogenic potential.

Materials and Methods

Cell lines. The Human T-ALL cell line Loucy (control and with CASZ1b overexpression) was maintained in RPMI medium supplemented with 10% FBS (RPMI-10) and split every 2-3 days. The murine cell line BaF3 (control and with CASZ1b overexpression) was maintained in RPMI10 and supplemented with 2% conditioned media from WEHI-3B, a myelomonocytic leukemia cell line that secretes IL3.

Western blot. Cell lysates were prepared, and equal amounts of protein were separated by 12% SDS-polyacrylamide electrophoresis. The proteins were transferred onto nitrocellulose membranes and immunoblotted with the antibodies described below. After immunoblotting with the primary antibody, immunodetection was performed using HRP-conjugated anti-mouse or anti-rabbit IgG with appropriate reagent for detection.

Cell Survival and growth. Determination of cell viability was performed by flow cytometry analysis of forward scatter (FSC) versus side scatter (SSC) distribution using a LSR Fortessa-2 flow cytometry (Becton–Dickinson, Mountain View, CA, USA). The host laboratory previously confirmed that this strategy accurately measures lymphoid cell viability. Cell counts were determined by extrapolating the number of live cells (within the FSC/SSC gate described above) acquired in a fixed volume (40µl) that is analyzed in the flow cytometer Highthroughput Sampler (HTS).

Statistical analysis. Differences between populations were calculated using either a One-way or Two-way ANOVA, when appropriate ($p<0.05$ was considered significant).

Results

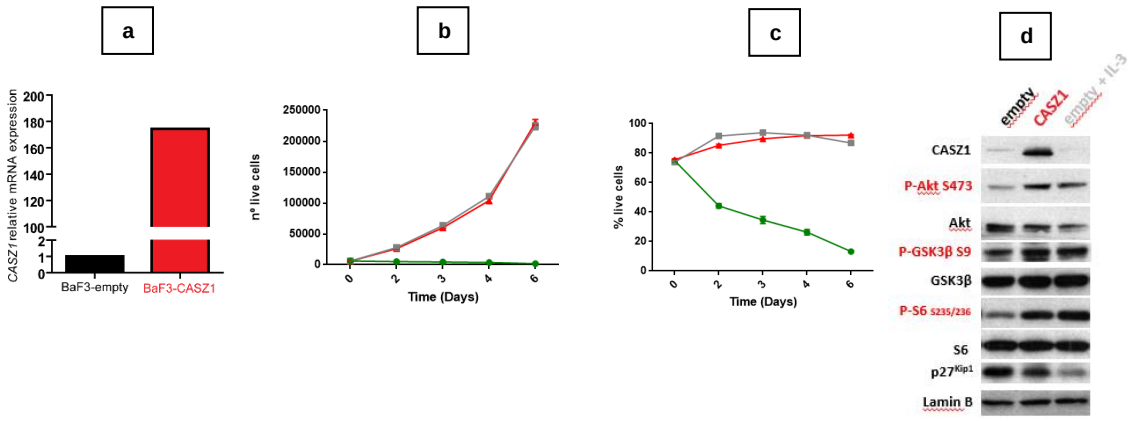

Figure 1. ***CASZ1b* promotes cell survival and cell growth via PI3K-Akt signalling pathway.** BaF3 cell experiment under three conditions – empty (*CASZ1b*-) without IL3 (green); *CASZ1b* without IL3 (red); empty with IL3 (grey). **(a)** *CASZ1b* mRNA relative expression. Cell growth **(b)** and cell viability **(c)** on a 6-day time-course experiment. **(d)** Western-blot showing the phosphorylated and total protein levels of the indicated PI3K-Akt signalling effectors.

As shown in figure 1 (a – c) CASZ1b overexpression in BaF3 cells, an IL3-dependent murine cell line, mimics the effect of IL3 on cell survival and cell growth. This shows that CASZ1b transforms growth factor-dependent cells and makes them become growth factor-independent. This is the behavior of an oncogene. This effect on cell survival and cell growth of CASZ1b was concomitant with the activation of the PI3K-Akt signaling, as shown by the increased phosphorylation of its downstream effectors like Akt, GSK3β and S6 (figure 1d).

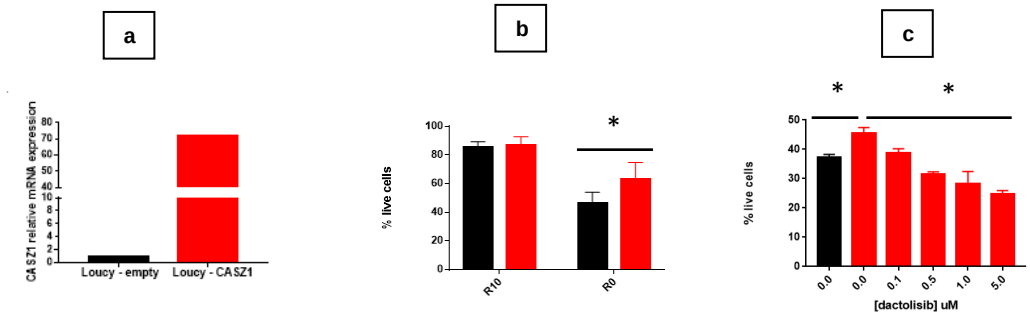

Figure 2. *CASZ1b* overpression rescues T-ALL cell viability. (a) *CASZ1* mRNA relative expression. **(b)** Loucy cell survival experiment in complete medium (R10) or in serum-deprived (R0) conditions at 24h. **(c)** Loucy cells cultured with Dactolisib in R0 conditions at 24h. (**red** – *CASZ1*; **black** – empty) * p value < 0.05

As demonstrated in Figure 2b CASZ1b over-expression has no impact on the survival of Loucy cells when compared to control (Empty) cells in regular medium (R10). However, CASZ1b has a statistically significance impact on viability under stress conditions (serum starvation). From figure 2c we can infer that PI3K signaling pathway inhibition with Dactolisib (a dual PI3K-mTOR inhibitor) might be important to decrease the viability and proliferation of a T-ALL cell line over-expressing CASZ1. CASZ1 improves viability (compare first and second column), however, as we increase dactolisib's concentration in the medium the oncogenic potential of CASZ1 is further and further abolished. On figure 2c, two-way ANOVA multiple comparison tests between all concentrations of dactolisib (columns 3, 4, 5 and 6) and R0 CASZ1 + (column 2) were statistically significant as between R0 with and without CASZ1 (column 1).

Considering the transcriptomic data, the BaF3 experiments (figure 2d) and these results, it is fair to assume that at least part of the potential oncogenic effect of CASZ1b is via the PI3K signaling pathway.

Conclusions

Our experiments have shown that CASZ1b transforms BaF3 cells and this associates with activation of PI3K-Akt signalling pathway in a murine cell line – BaF3. With this in mind, a dual PI3K-mTOR inhibitor – dactolisb – was tested in a human T-ALL cell line with overexpression of CASZ1. Our results suggest that Dactolisib might be useful to promote the death of Loucy cells with CASZ1 overexpression.

LIMITATIONS

The current review and scientific experiment are believed to have achieved the proposed goals in a complementary way. Despite this fact, the review could have benefited from including a deeper analysis of the TFs that may act as tumour suppressors, such as RUNX1 (also behaves as an oncogene), GATA3, LEF1 and ETV6. Even though we believe this thorough analysis would have deviated us from the main target, they were mentioned along the way, apart from ETV6. Regarding the scientific experiment, to better characterize the impact of CASZ1 on T-ALL biology, we believe *in*

vivo models and a characterization of CASZ1 transcriptional program could be future steps.

FUTURE DIRECTIONS

The main statement this work ought to make is that a deeper understanding of the genetic programs underlying the development and maintenance of T-ALL will allow more rational and tailored therapeutics in T-ALL depending on its subgroups.

Undoubtedly, the future will go in this direction, since even though T-ALL is one disease, the paths that lead T-cells to the same ending (blasts) are genetically distinct. *In vivo* models that allow testing of new therapeutics before applying them to humans could have a greater development and implementation in research and then in clinical practice.

Toxic and conventional therapies like chemotherapy with the previously introduced – L-asparaginase, cyclophosphamide, vincristine and anthracycline, will tend to be substituted, or at least complemented, by targeted therapies that may hopefully increase survival and decrease toxicity. The PI3K pathway emerges as a new and rational target that is why PI3K pharmacological inhibitors have ongoing clinical trials (CT), such as everolimus+HyperCVAD (CT identifier NCT00968253) chemotherapy and everolimus+multi-agent chemotherapy (CT identifier NCT01523977).

Initially a pathologist in a hospital cellar, Sidney Farber became the father of chemotherapy through pioneering leukemia research and fundraising with Mary Lasker. Dr. Farber has inspired generations and proved that with big dreams, small players can achieve greatness. *CASZ1* and *TAL1* may be small genes in hematology genetics, however, they have knocked on the doors of a brave new world of targeted therapeutic rationality. Maybe someday we can prove Voltaire wrong, but the time has not come yet.

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