Cancer Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Towards precision medicine in childhood leukemia – Insights from mutationally activated cytokine receptor pathways in acute lymphoblastic leukemia

Shai Izraeli [a,b,*], Chen Shochat [a,b,c,d], Noa Tal [a,b], Ifat Geron [a,b,e]

[a] Childhood Leukemia Research Section, Department of Pediatric Hemato-Oncology, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel
[b] Department of Molecular Human Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[c] Migal Galilee Technology Center, Kiryat Shmona, Israel
[d] Tel Hai College, Upper Galilee 12210, Israel
[e] Division of Biological Sciences and Department of Medicine Stem Cell Program, University of California San Diego, La Jolla, CA, USA

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Acute lymphoblastic leukemia
CRLF2
Interleukin 7 receptor
Risk stratification

ABSTRACT

The successful therapy of childhood leukemia has been characterized by careful personalized adaptation of therapy by risk stratification. Yet almost all drugs are relatively non-specific. To achieve greater precision in therapy, druggable targets and specific targeting drugs are necessary. Here we review the recent discoveries of cytokine receptors and their signaling components in high risk leukemias and the potential approaches to target them.

1. Introduction

Treatment of children with acute lymphoblastic leukemia (ALL) has long been personalized and adjusted to a variety of risk factors (Table 1) [1–3]. These risk factors consist of parameters such as age, clinical presentation of the leukemia including the immunophenotype, white cell count and the cytogenetic subgroup. For example the presence of a chromosomal translocation fusing the ETV6 gene on chromosome 12 with the RUNX1 gene on chromosome 21 is associated with excellent prognosis while the presence of genomic rearrangements involving the MLL gene on chromosome 11 are indicators of bad prognosis.

Based on these initial diagnostic parameters treatment is adjusted by risk classification, usually into 2–4 subgroups. This classification is further refined by observing the initial response of leukemia to therapy determined by the level of minimal residual disease (MRD) with sensitive means such as flow cytometry by molecular quantification of leukemia specific markers [4–7].

Yet despite this careful adaptation of therapy to the risk of relapse, the drugs used for therapy have hardly changed over the last decades, and in general do not specifically target the genetic abnormality driving the leukemic clone. Drugs immediate and long term toxicities (a factor specifically important in children) are substantial and additional non-specific intensification of therapy is unlikely to further raise cure rates of high risk and relapsed leukemias. The challenge is to develop a "precision medicine" approach by which current non-specific therapy is replaced, or supplemented, by highly targeted therapy to the specific subtype of leukemia [8].

One recent example of such precision medicine approach is the incorporation of ABL1 kinase inhibitors into the treatment of *BCR-ABL1* positive ALL [9]. The *BCR-ABL1* fusion is caused by a translocation between chromosome 9 and 22, resulting in the "Philadelphia Chromosome" and the formation of a constitutively active BCR-ABL1 kinase. While uncommon, BCR-ABL1 positive ALLs have been associated with a particular bad prognosis and have been regarded as a clear indication for stem cell transplantation [10]. Nevertheless, the addition of the tyrosine kinase inhibitor Imatinib improved dramatically the outcome of these leukemias [9,11,12].

Here we review another genetic aberration associated with bad prognosis that is a potential target to such precision medicine – the aberrant expression of the cytokine receptor CRLF2 and the mutational activation of the CRLF2-IL7R-JAK-STAT signaling pathway in B cell precursor ALL, the most common type of ALL. Recent research has also indicated that the *CRLF2* aberration is one of several abnormalities that characterize a high risk group of childhood and adult leukemias and might be amenable to targeted therapy.

* Corresponding author at: Sheba Medical Center, Tel-Hashomer, Ramat Gan 52621, Israel. Tel.: +972 3 530 3037.
E-mail address: Shai.Izraeli@sheba.health.gov.il (S. Izraeli).

http://dx.doi.org/10.1016/j.canlet.2014.02.009
0304-3835/

Table 1
Risk factors used for "personalized" adaptation of treatment for childhood ALL[a].

Demographic factors	Age
	Gender (in some protocols affect length of maintenance therapy)
	Underlying disease (e.g. Down Syndrome)
Leukemia factors	White cell count at presentation
	Immunophenotype
Cytogenetic subgroups	"Good Risk" ETV6-RUNX1; Hyperdiploidy
	"Bad Risk" MLL translocations, BCR-ABL, Hypodiploidy
Response to therapy	Morphological response
	Minimal residual disease by PCR or flow cytometry

[a] Not all protocols utilizes all risk factors.

2. A Lymphoid specific JAK2 activating mutation – collaboration with a lymphoid specific receptor?

The JAK kinases play important roles in transmitting signals from non tyrosine kinases cytokine receptors [13]. It has been shown that activating mutations in JAK2, most commonly V617F, are present in the majority of myeloproliferative neoplasms [14–17]. The constitutive activation of this enzyme by a mutation in the regulatory "pseudokinase domain" abrogates the dependency of erythroid and megakaryocytic precursor on erythropoietin and thrombopoietin respectively and enhances their proliferation causing polythytemia and thromocytosis respectively. This exciting discovery has initiated screening for JAK mutations in a variety of hematopoietic neoplasms. Screening 90 cases of childhood ALL, Maligne et al. [18] identified a single specimen with a deletion of four amino acids at position 683 in the pseudokinase domain of JAK2 causing its constitutive activation. This patient had also Down Syndrome (DS).

Children with DS are at a markedly increased risk for developing ALL (DS-ALL) that is 20 times more common compared to children without DS [19]. Further studies confirmed the presence of activating JAK2 mutation in up to 20% of DS-ALL compared with 1–3% of ALL in children without DS [20–24].

Interestingly, most of the mutations replace or are localized within the immediate vicinity of Arginine at position 683. They are B-lymphoid specific and have never been described in myeloproliferative neoplasms. Conversely the V617F mutation has never been described in ALL. Moreover, the V617F is present in hematopoietic stem cell, yet the lymphoid compartment is normal in patients with MPNs [25,26]. Although V617 and R683 are present at different areas of the pseudokinase domain [20,27] the biochemical basis for the exquisite lineage specificity of these somatic mutations is unknown. Modeling in mice has failed so far to replicate the human differential mutation phenomenon – expression of either mutated JAK2 allele in hematopoietic stem cell causes a myeloproliferative neoplasm [18].

For induction of signaling, binding of JAK2 to the cytosolic component of a cytokine receptor is required. Hence, it was reasonable to hypothesize that R683 mutated JAK2 interacts with a lymphoid specific receptor in B-ALL. This receptor proved to be CRLF2.

3. CRLF2. – "Hijacking" of a receptor

The *CRLF2* gene resides on the pseudoautosomal region of the sex chromosomes Xp22.3 and Yp11.3. It encodes an atypical type 1 non tyrosine kinase cytokine receptor that is most similar to IL2RG, the partner of IL7RA in the receptor to IL7. CRLF2 heterodimerizes with IL7RA and forms the receptor for Thymic Stromal Lymphopoietic (TSLP) [28–30]. TSLP is a cytokine secreted from bronchial and intestinal epithelial cells, keratinocytes and other stromal cells. It mediates inflammatory and allergic reactions and its levels are increased in asthma and atopic dermatitis [31–34]. The heterodimeric TSLP receptor comprised from CRLF2 and IL7RA is normally expressed in some CD4 cells, dendritic cells, basophils and on the newly discovered innate lymphoid cells [35–37]. It is not expressed in a significant level in human B cell precursors.

Aberrant expression of CRLF2 is present in about 60% of DS-ALLs and in up to 10% of B cell precursor ALLs in children or adults [38–41]. This expression is usually caused by genomic rearrangements. In children the most common aberration is a micro-deletion on chromosome X or Y that creates a fusion between the first non-coding exon P2RY8, a constitutively expressed gene and CRLF2. Alternatively, more common in adults, CRLF2 is translocated into the IgH enhancer locus on chromosome 14 [38–40]. The presence of these aberrations is associated with worse prognosis as shown in analysis of many clinical trials of ALL, although the impact on prognosis depends on the specific treatment protocol [41–50] and on associated genetic aberrations in the *IKZF1* gene [51].

The abnormally expressed CRLF2 form with IL7RA the receptor to TSLP. Indeed it has been recently shown that leukemic blasts from primary ALLs with overexpression of CRLF2 respond to TSLP by phosphorylation of the downstream signaling molecules JAK2, JAK1, STAT5 and SP6 [52]. So presumably pre-leukemic cells misexpressing the CRLF2 receptor can respond to TSLP secreted from the bone marrow stroma. Direct experimental evidence to this hypothesis is lacking. Furthermore as discussed below, additional mutations in this pathway seems to be required for the leukemogenic process.

4. Mutational activation of the CRLF2-IL7RA (TSLP) pathway

Strikingly the majority of the CRLF2 expressing leukemias carry also somatic activating mutations of this pathway. Virtually all cases with the JAK2 R683 mutations described are present in cells aberrantly expressing CRLF2. Co-expression of both proteins is both sufficient and required for leukemic transformation of cytokine dependent mouse pro-B cells (Fig. 1) [38–41] (and Tal and Izraeli, unpublished data).

Yet, JAK2 mutations (also in sites outside arginine 683, but never the V617F myeloid mutation) are present in no more than a third of CRLF2 positive ALL. Accordingly, other mutations activating the signaling pathway have been discovered. CRLF2 binds to JAK2 and IL7RA interacts with JAK1. Thus it was not surprising that activating JAK1 mutations in CRLF2 positive ALL were also discovered [21,53].

Another approach for activation of a pathway may be through inactivation of a negative regulator. Indeed, biallelic deletions or loss of function mutations in *SH2B3* were recently reported in some CRLF2 positive leukemias [54]. SH2B3 encodes the adaptor protein LNK that inhibits the activity of JAK2 [55–58]. LNK is an important regulator of lymphopoiesis in mice [59,60]. Interestingly two siblings with an autoimmune disorder and B cell ALL and a germline mutation in *SH2B3* were recently reported [61].

Recently novel receptor activating mutations were discovered in CRLF2 positive leukemias lacking a mutation in the signaling components of the TSLP pathway [38,41,62]. These mutations occurring either in CRLF2 or in it partner IL7RA most commonly introduce a cystein into the juxtamembrane domain of the receptor. In the oxidative extracellular environment this cystein causes homodimerization of mutated receptor through creation of S-S bonds. The covalently dimeric receptor signals constitutively and transforms mouse B cell progenitors in vitro and in vivo (Tal and Izraeli, unpublished). Mutational activation of the IL7RA occurs also in the absence of CRLF2 in B and in 10% of T cell ALL [54, 62–64].

Please cite this article in press as: S. Izraeli et al., Towards precision medicine in childhood leukemia – Insights from mutationally activated cytokine receptor pathways in acute lymphoblastic leukemia, Cancer Lett. (2014), http://dx.doi.org/10.1016/j.canlet.2014.02.009