Cellular Signalling xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Cellular Signalling

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

Review

New insights into Notch1 regulation of the PI3K–AKT–mTOR1 signaling axis: Targeted therapy of γ-secretase inhibitor resistant T-cell acute lymphoblastic leukemia

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ARTICLE INFO

Article history:
Received 20 September 2013
Accepted 30 September 2013
Available online xxxx

Keywords:
Notch1
AKT
mTOR
Protein phosphatase 2A
γ-Secretase inhibitor resistance
Leukemia

ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) is characterized as a high-risk stratified disease associated with frequent relapse, chemotherapy resistance, and a poorer prognostic outlook than B-precursor ALL. Many of the challenges in treating T-ALL reflect the lack of prognostic cytogenetic or molecular abnormalities on which to base therapy, including targeted therapy. Notch1 activating mutations were identified in more than 50% of T-ALL cases and can be therapeutically targeted with γ-secretase inhibitors (GSIs). Mutant Notch1 can activate cMyc and PI3K–AKT–mTOR1 signaling in T-ALL. In T-ALLs with wild-type phosphatase and tensin homolog deleted on chromosome ten (PTEN), Notch1 transcriptionally represses PTEN, an effect reversible by GSIs. Notch1 also promotes growth factor receptor (IGF1R and IL7Rα) signaling to PI3K–AKT. Loss of PTEN is common in primary T-ALLs due to mutation or posttranslational inactivation and results in chronic activation of PI3K–AKT–mTOR1 signaling, GSI-resistance, and repression of p53-mediated apoptosis. Notch1 itself might regulate posttranslational inactivation of PTEN. PP2A is activated by Notch1 in PTEN-null T-ALL cells, and GSIs reduce PP2A activity and increase phosphorylation of AKT, AMPK, and p70S6K. This review focuses on the central role of the PI3K–AKT–mTOR1 signaling in T-ALL, including its regulation by Notch1 and potential therapeutic interventions, with emphasis on GSI-resistant T-ALL.

Contents

Abbreviations: ADAM, a disintegrin and metalloprotease; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AKT, protein kinase B (PKB); ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AMPK, adenosine monophosphate (AMP) activated protein kinase; ANK, ankyrin-like repeats; BAD, BCL-2 associated death promoter; Bim, BCL-2 homology domain 3 (BH3)-only protein, B-cell lymphoma 2 interacting mediator of cell death; BP-ALL, B-precursor acute lymphoblastic leukemia; CAMKKβ, calmodulin-dependent protein kinase kinase beta; CDK8, cyclin-dependent kinase 8; CK2, casein kinase 2; CLL, B-cell chronic lymphocytic leukemia; CSL, CBF1/Su(H)/Lag-1; Dll, Delta-like; DSL, Delta–Serrate–Lag1; EFS, event free survival; EGF, epidermal growth factor; eIF2A and eIF4E, eukaryotic translation initiating factors 2A and 4E; ERK, extracellular signal-regulated protein kinase; FBW7, F-box/WD-repeat containing protein 7; FOXO, fork head box O transcription factors; GSC, γ-secretase complex; GSI, γ-secretase complex inhibitors; GSK3, glycogen synthase kinase 3; HD, heterodimerization domain; Hes1, hairy and enhancer of split-1; ICN1, intracellular domain of Notch1; IGF1R, insulin-like growth factor 1 receptor; IGFBP3, insulin-like growth factor-binding protein 3; IL7Rα, interleukin-7 receptor subunit α; IRS1, insulin receptor substrate 1; Jag, Jagged; JME, juxtamembrane expansion; LKB1, liver kinase B1; LNR, Lin12/Notch1 repeats; MAML1, mastermind-like protein 1; MAPK, mitogen activate protein kinases; MCL1, induced myeloid leukemia cell differentiation protein; MDM2, mouse double minute 2 homolog; miR/miRNA, micro-RNA; mSIN1, mammalian stress-activated protein kinase interacting protein 1; mTOR, mammalian target of rapamycin; NEC, Notch1 extracellular domain; NTM, Notch1 transmembrane domain; PDK1, phosphoinositide dependent protein kinase-1; PEST, Pro, Glu, Ser, and Thr-rich domain; PI3K, phosphatidylinositide 3-kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKCθ, protein kinase C theta; PP2A, Ser/Thr-protein phosphatase 2A; PRAS40, proline-rich AKT substrate 40 kDa; PTEN, phosphatase and tensin homolog deleted on chromosome ten; raptor, regulatory-associated protein of mTOR; rictor, rapamycin-insensitive companion of mTOR; ROS, reactive oxygen species; RUNX1 and RUNX3, runt-related transcription factors 1 and 3; T-ALL, T-cell acute lymphoblastic leukemia; TACE, tumor necrosis factor α-converting enzyme; TAN1, translocation associated Notch1 homolog.

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0898-6568/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.cellsig.2013.09.021

1. Biology and therapy of T-cell acute lymphoblastic leukemia

T-cell acute lymphoblastic leukemia (ALL) accounts for 10–15% of pediatric and 25% of adult ALL cases [1,2]. In recent years, treatment of pediatric T-ALL has significantly improved with 5-year event-free survivals (EFS) of 70–75%, approaching that for B-precursor (BP) ALL in children [1,3,4]. However, T-ALL in adults remains an aggressive disease with 5-year EFS of 20–50% [5]. Early relapse is common in T-ALL and is associated with an extremely poor prognosis [6]. Early T-cell precursor ALL is also refractory to treatment [7].

In spite of the increased EFS for T-ALL patients, particularly children, toxicity to standard chemotherapy continues to present a major challenge. For instance, intensive treatment strategies for T-ALL, such as the use of glucocorticoids and anthracycline antibiotics (i.e., doxorubicin), are associated with significant acute and longer-term toxicities [8,9]. For central nervous system disease, the use of cranial irradiation, which has severe long-term debilitating effects, has been largely replaced with intrathecal and systemic treatments, albeit with their own toxicities [10]. Clearly, there is a need for improved treatment strategies for T-ALL with reduced overt and long-term toxicities.

Much of the success in treating BP-ALL in children reflects the identification of subgroups of patients whose disease shows distinct cytogenetic or molecular abnormalities [e.g., hyperdiploidy and t(12;21) translocation] on which to base treatment [11]. T-ALL is a heterogeneous disease that is typically associated with fewer unique features than BP-ALL upon which to stratify patients, even though specific non-random translocations have been identified [11].

The most common genetic alteration in T-ALL involves deletion of the CDKN2A/2B locus including the $p16^{INK4A}$, $p14^{ARF}$, and $p15^{INK4B}$ tumor suppressor genes [12,13]. The most common chromosomal translocations in T-ALL juxtapose promoter/enhancer elements of the T-cell receptor (TCR) genes (TCRβ at 7q32-q26 and TCRα/TCRδ at 14q11) to oncogenic transcription factor genes including cMYC, HOX11, TAL1, LYL1, LMO1 and LMO2, resulting in over-expression of downstream gene targets [14]. The MLL–ENL fusion gene results from t(11;19)(q23;q13) [15]. Nearly all T-ALLs can be grouped into subtypes, based on the effects of chromosomal translocations on gene expression profiles. These include HOX11L2, LYL1 plus LMO2, TAL1 plus LMO1 or LMO2, HOX11, and MLL-ENL. The HOX11 and MLL–ENL subtypes are associated with favorable prognoses, whereas HOX11L2 confers a worse prognosis [11].

Notch designates a family (Notch1–4) of heterodimeric transmembrane receptors that regulate cell differentiation, proliferation, and apoptosis, and play a critical role in development [16]. Notch1 signaling is required for a commitment of pluripotent progenitors to a T-cell fate and normal T-cell development [17]. Notch1 was discovered as a TCRβ partner gene (termed "trans-activation domain of Notch1" or TAN1) involving the t(7;9) (q23;q34.3) translocation in a patient with T-ALL [18]. t(7;9) is associated with constitutively active Notch1 that results in downstream effects on transcription of target genes [19]. T-ALL cells require constitutively active Notch1 signaling for cell proliferation [20–23]. Although t(7;9) occurs in less than 1% of T-ALLs, a wider, oncogenic role for Notch1 was suggested by reports that insertion of constitutively active Notch1 into bone marrow progenitors transplanted into syngeneic mice induced T-ALL [24]. Moreover, Notch1 activating mutations were reported in greater than 50% of T-ALL cases and mutations in the gene encoding Notch1-related FBW7 (F-box/WD-repeat containing protein 7) (Sel10) ubiquitin ligase were reported in 8–16% of T-ALLs [23,25,26]. Collectively, these findings suggest that aberrant Notch1 signaling is linked to the pathogenesis of T-ALL.

Growing evidence demonstrates that Notch1 regulates PI3K–AKT–mTOR1 signaling in T-ALL, although many of the details are still emerging. The phosphatase and tensin homolog deleted on chromosome ten (PTEN) antagonizes activation of the Ser/Thr kinase AKT (PKB) [27]. Notch1 represses PTEN, activating AKT signaling [22]. Very recent studies identified the Ser/Thr protein phosphatase 2A (PP2A) as a novel regulator of PI3K–AKT signaling and a downstream target of Notch1 [28]. Activation of AKT signaling can cooperate with Notch1 to promote leukemogenesis or growth of established leukemia, and relieve dependence of cell proliferation on Notch1 signaling [22]. The mammalian target of rapamycin complex 1 (mTOR1) pathway is a major point of convergence for Notch1 and PI3K–AKT signaling and promotes growth of T-ALL cells [20,22,29]. AKT and/or mTOR1 inhibitors are potent against T-ALLs with activated Notch1, further evidence that the PI3K–AKT–mTOR1 signaling axis is an important conduit for the effects of Notch1 signaling on T-ALL cell survival [30]. In the following sections, we explore the complex interplay between Notch1 and PI3K–AKT–mTOR1 signaling as a prelude to better exploiting these critical pathways for improved therapy of T-ALL.

2. The Notch1 signaling pathway

The mature Notch1 transmembrane receptor (Fig. 1) consists of an extracellular (NEC) domain and a transmembrane (NTM) domain, which harbors the Notch1 intracellular (ICN1) domain. Pro-Notch1 is expressed, as a single polypeptide that is cleaved at its S1 site by a furin-like convertase to create a heterodimerization (HD) domain comprised of non-covalently associated NEC and NTM domains [31,32]. The NEC domain consists of thirty-six epidermal growth factor (EGF)-like repeats, followed by three Lin12/Notch1 repeats (LNR) and the HD domain. The latter creates a negative regulatory region (LNR-HD) that prevents promiscuous cleavage of the S2 cleavage site by an ADAM (a disintegrin and metalloprotease) protease (formally, TACE) [17,33,34]. The NTM domain also includes the γ-secretase complex (GSC) S3 cleavage site [35]. ICN1 includes a RAM domain, seven tandem ankyrin-like repeats (ANK), flanked by nuclear localization signals, and a transcription activation domain [17]. ICN1 also harbors a carboxyl terminal Pro, Glu, Ser, and Thr-rich (PEST) domain that regulates ICN1 turnover [36].

S1 cleavage of pro-Notch1 within the Golgi apparatus facilitates Notch1 heterodimer formation [31,32] (Fig. 2A). Fringe glycosyltransferases modify the Notch1 EGF-like repeats (Fig. 2A) to regulate ligand specificity and subsequent proteolysis at the plasma membrane [37]. Binding one of five DSL (Delta–Serrate–Lag-2) ligands [Serrate-like Jagged- (Jag-) 1 and 2, and Delta-like- (Dll-) 1, 3, and 4 in humans], expressed on the surface of neighboring cells, to the EGF-like repeats triggers cleavage by an ADAM protease at the S2 site [17,33,38,39] (Fig. 2B). This Notch1 activation process is facilitated by ubiquitination of DSL ligands by the E3-ubiquitin ligases, mind bomb and neuralized, resulting in ligand internalization and subsequent proteolysis [40,41]. Ligand internalization has been suggested to physically dissociate the NEC domain from the NTM domain, exposing the S2 site to

Please cite this article as: E.C. Hales, et al., Cell. Signal. (2013), http://dx.doi.org/10.1016/j.cellsig.2013.09.021