Targeting Interleukin-2-Inducible T-cell Kinase (ITK) in T-Cell Related Diseases

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Abstract
IL2-inducible T-cell kinase (ITK), a member of the Tec family tyrosine kinases, is the predominant Tec kinase in T cells and natural killer (NK) cells mediating T cell receptor (TCR) and Fc receptor (Fc R) initiated signal transduction. ITK deficiency results in impaired T and NK cell functions, leading to various disorders including malignancies, inflammation, and autoimmune diseases. In this mini-review, the role of ITK in T cell signaling and the development of small molecule inhibitors of ITK for the treatment of T-cell related disorders is examined.

Keywords: IL2-inducible T-cell kinase (ITK), inhibitors of ITK, T cell signaling

Introduction

The Tec (tyrosine kinase expressed in hepatocellular carcinoma) family tyrosine kinases play important roles in mediating intracellular signaling in hematopoietic cells [1]. They consist of five members: Tec, Bruton's tyrosine kinase (BTK), IL2-inducible T-cell kinase (ITK, also known as EMT or TSK), resting lymphocyte kinase (RLK, also known as TXK) and bone marrow-expressed kinase (BMX, also known as ETK). BTK is an essential regulator of B cell receptor (BCR)-initiated signaling pathways [2, 3]. Three Tec kinases including ITK, RLK and TEC are expressed in T lymphocytes. ITK is expressed at the highest level in naïve T cells and thymocytes, followed by RLK and then TEC [4]. Their expression levels possibly contribute to their relative importance in T cells. $Itk^{-/-}$ mice have profound defects in T-cell development and function; combined deletion of ITK and RLK worsens these defects [5-7]; however, no major defects in T cells have been described in $Tec^{-/-}$ mice [8]. During $CD4^{+}$ T cell differentiation, ITK is expressed in both T helper type 1 (Th1) and T helper type 2 (Th2) cells with an upregulated level in Th2 cells, while RLK is expressed only in Th1 cells but not in Th2 cells, suggesting a critical role of ITK in the differentiation and function of Th2 cells [9]. ITK has also been shown to be important for the development of invariant natural killer T (NKT) αβ cells and NKT-like γδ T cells besides conventional T-cells [10, 11]. In natural killer (NK) cells, ITK positively regulates Fc receptor (FcR) - induced granule release, calcium mobilization, and cytotoxicity but negatively regulates natural killer group 2, member D (NKG2D)-initiated signaling [12]. These studies implicate an essential role of ITK in T and NK cell development and function, and therefore developing small molecular inhibitors of ITK for treatment of T and NK cell related diseases would be attractive.

Domain structure of ITK

The ITK gene, discovered in 1992 [13, 14], is chromosomally localized at 5q31-32 position in humans [15]. ITK protein is a 72 kDa kinase expressed in T cells, NK cells and mast cells, and it contains five distinct domains including pleckstrin homology (PH), Tec homology (TH), Src homology 3 (SH3), Src homology 2 (SH2) and Src homology 1 (SH1) (Figure 1) [16, 17]. The PH domain is at the amino terminal of ITK and helps the protein to bind phosphorylated lipids on the membrane [18, 19]. The TH domain exhibits the familial recognition of ITK and contains a proline rich region (PRR) which is

necessary for binding the SH3 domain [20, 21]. The SH3 domain binds to the PRR in the TH domain, resulting in the auto-inhibited state of ITK, while the SH2 domain enables protein-protein interactions and allows ITK to bind with phospholipase Cγ1 (PLCγ1) [22-25]. Therefore, ITK is activated by mutations or deletions in the SH3 domain and inactivated by mutations and deletions in the SH2 domain. At the carboxyl terminus of ITK is the SH1 domain which contains ITK's kinase activity resides and adenosine 5'-triphosphate (ATP) binding pocket [26]. Known targets of this domain include PLCγ1 [27], T-bet [28], Tim-3 [29] and TFII-I [30].

Figure 1. Domain structure of ITK. ITK protein contains five distinct domains including pleckstrin homology (PH), Tec homology (TH), Src homology 3 (SH3), Src homology 2 (SH2) and Src homology 1 (SH1)

ITK-mediated TCR signaling pathways

ITK is a critical mediator of T cell receptor (TCR) signaling (Figure 2). Upon TCR stimulation, the TCR interacts with peptide-MHC complex presented on antigen presenting cells, leading to the activation of lymphocyte-specific protein tyrosine kinase (LCK) and phosphatidylinositol 3-kinase (PI3K), two important molecules for the activation of ITK [26, 31, 32]. Activation of LCK results in phosphorylation of the CD3 immunoreceptor tyrosine-based activation motifs (ITAMs) and the recruitment of Zeta-chain-associated protein kinase 70 (ZAP70) to activated ITAMs [33], and then these two kinases phosphorylate downstream adaptors linker for activation of T cells (LAT) and SH2 domain-containing leukocyte protein of 76 kDa (SLP-76) [34, 35]. Following PI3K activation, ITK is brought from the cytoplasm to the plasma membrane via the interaction between its PH domain and the PI3K phosphorylated phospholipids in the plasma membrane [36, 37]. There, ITK interacts with the activated complex of SLP-76 and LAT adaptors via its SH2 and SH3 domains, leading to its phosphorylation on the activation loop (Y511) by Lck [26, 38-40].

Activated ITK autophosphorylates Y180 in its SH3 domain and phosphorylates its downstream target PLCγ1 (Figure 2) [41, 42]. Autophosphorylated Y180 modulates the binding of ITK to different protein targets. Activated PLCγ1 hydrolyzes phosphatidylinositol biphosphate (PIP2) to produce inositol-3-phosphate (IP3) and diacylglycerol (DAG), two critical second messengers in TCR signaling [43]. IP3 binds to receptors on intracellular organelles and causes Ca^{2+} release from the intracellular store, leading to sustained Ca^{2+} influx and downstream activation of transcription factors including nuclear factor of activated T-cells (NFAT) [6, 7, 44]. DAG activates two signal pathways. One is the mitogen activated protein kinase (MAPK) pathway which leads to the activation of extracellular signal regulated kinase (ERK) and the other is the Protein Kinase C (PKC) pathway which activates nuclear factor-kappaB (NF-κB) and c-Jun amino-terminal kinase (JNK) [45, 46].

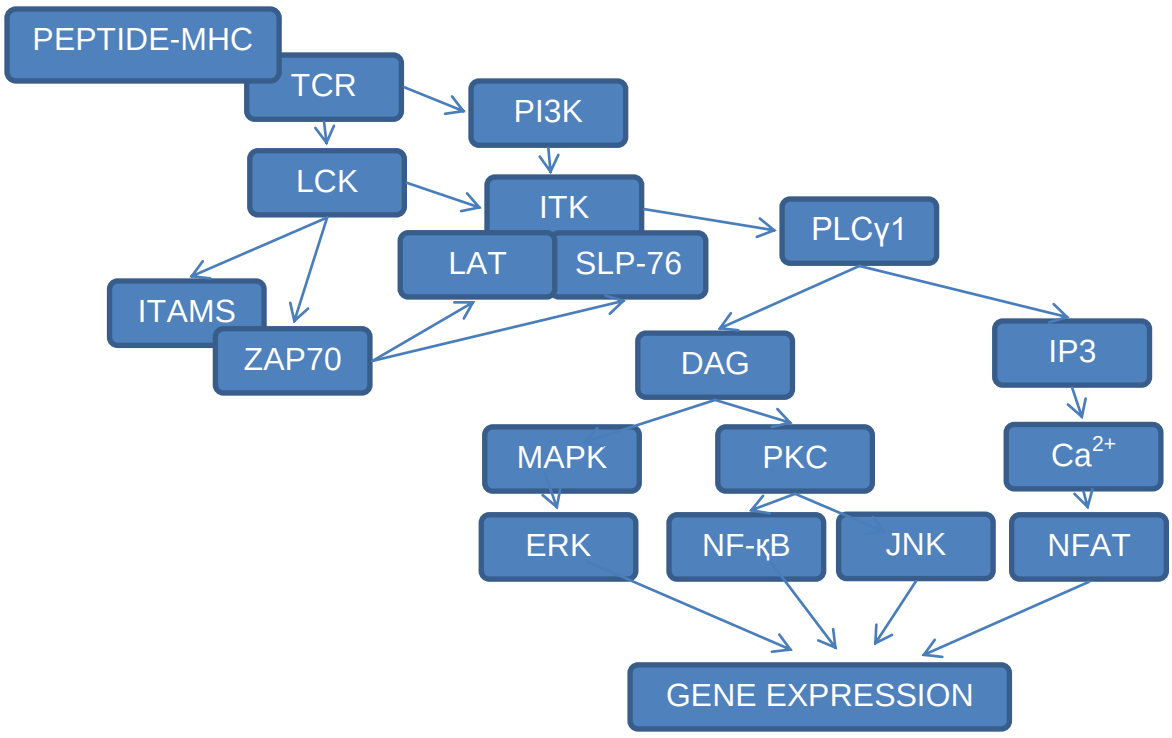

Figure 2. ITK-mediated T cell receptor (TCR) signaling pathway. Upon TCR stimulation, TCR interacts with peptide-MHC complex presented on antigen presenting cells, leading to the activation of LCK and PI3K. Activation of LCK results in phosphorylation of the CD3 immunoreceptor tyrosine-based activation motifs (ITAMs) as well as ZAP70 and downstream adaptors LAT and SLP-76. Following PI3K activation, ITK is brought from the cytoplasm to the plasma membrane. There, ITK interacts with the activated complex of SLP-76 and LAT adaptors, leading to its phophorylation on the activation loop (Y511) by Lck. Activated ITK autophosphorylates Y180 in its SH3 domain and phosphorylates its downstream target PLCγ1. Activated PLCγ1 hydrolyzes PIP2 to produce inositol-3-phosphate (IP3) and diacylglycerol (DAG). IP3 binds to receptors on intracellular organelles and cause Ca^{2+} release from the intracellular store, leading to sustained Ca^{2+} influx and downstream activation of transcription factors including NFAT. DAG activates two signal pathways. One is mitogen activated protein kinase (MAPK) pathway which leads to the activation of extracellular signal regulated kinase (ERK) and the other one is Protein Kinase C (PKC). pathway which activates nuclear factor-kappaB (NF-κB) and c-Jun amino-terminal kinase (JNK).

The role of ITK in T cell development and differentiation

Numerous studies have shown that ITK deficiency results in defects in T cell development. Itk knockout mice have impaired T cell activation, decreased numbers of mature thymocytes, reduced cell proliferation, lower ratios of $CD4^+$:$CD8^+$ T cells, and defects in thymic selection [5, 7, 44]. These phenotypes are worsened in Itk/Rlk double knockout mice, indicating functional compensation between these Tec kinases [7]. ITK deficiency affects not only conventional T lymphocytes but also innate T cells [47, 48]. Studies show that though total $CD4^+$ T cells and conventional $CD8^+$ T cells are reduced in $Itk^{-/-}$ mice, the total number of $CD8^+$ T cells remains normal due to the existence of innate $CD8^+$ T cells emanating from Itk knockout mice [49, 50].

ITK also regulates the differentiation of T helper cells including Th1, Th2, T helper 17 (Th17) and relatively normal levels of Th1 cytokines such as interferon gamma (IFNγ) but reduced Th2 cytokine including interleukin (IL)-4, IL-5 and IL-13, therefore, T cells in these mice preferentially develop into Th1 cells [9, 51, 52]. $Itk^{-/-}$ T cells also produce reduced IL-17A which regulates Th17 differentiation [53]. In addition, a new study identifies ITK as a critical regulator of the balance between Th17 and Treg cells by showing that $Itk^{-/-}$ $CD4^+$ T cells preferentially develop into Treg cells [54].

ITK and T cell disorders

Due to the critical role of ITK in T cell development and differentiation, disregulated ITK causes T cell related disorders.

Allergy and hypersensitivity

Itk knockout mice display reduced Th2 cells and Th2-type cytokines, and Th2 cells are important in the pathogenesis of inflammatory diseases including allergic asthma and atopic dermatitis, making ITK a potential therapeutic target in these diseases [55, 56]. Patients with allergic asthma have increased Th2 cells and Th2 cytokines which lead to lung inflammation [55]; Atopic dermatitis is caused by an excess of Th2 response and patients with atopic dermatitis have an increased expression level of ITK [57, 58].

Infection

Itk knockout mice have been shown to effectively clear parasites such as *Leishmania major*, *Nippostrongylus brasiliensis* and *Schistosome mansonii*. Infections with these pathogens elicit a strong Th2 response and are not cleared because of the lack of Th1 response. Mice without ITK display strong Th1 responses and produce normal levels of Th1 cytokines including IFN-γ, therefore, they efficiently clear the infections by these pathogens [7, 44, 51].

$Itk^{-/-}$ mice have increased memory like $CD8^+$ T cells with innate function, and thereby they have increased anti-bacterial responses to infection with *Listeria monocytogenes* [50]. However, Itk regulatory T cells (TReg). Itk deficient $CD4^+$ T cells produce deficiency also affects cytotoxic $CD8^+$ T cells, leading to impaired anti-viral immune responses [59, 60]. Itk mutations in the SH2 domain have been reported in patients with Epstein - Barr virus related lymphoproliferative diseases [61, 62], which may also be related to impaired cytotoxic $CD8^+$ T cell response.

Human immunodeficiency virus (HIV) is a retrovirus causing acquired immunodeficiency syndrome (AIDS). ITK has been shown to be an important factor in regulating the infection and replication of HIV [63]. Critical activators of HIV transcription including NFAT, NFκB and activator protein 1 (AP-1) are regulated by ITK [64]. In addition, the assembly and release of HIV viral particles is influenced by ITK via its regulation on actin cytoskeleton rearrangement [65-67].

Autoimmune diseases

Autoimmune diseases are caused by the activation of self-reactive T cells resulting in impaired organ function. ITK positively regulates Th17 cells which mediate autoimmune disorders including experimental autoimmune encephalomyelitis (EAE) [68, 69]. As a downstream mediator of the B7-CD28 co-stimulatory pathway, ITK also plays an important role in controlling the migration of auto-reactive T cells [70].

T cell malignancies

ITK has been shown to be up-regulated and aberrantly activated in T-cell malignancies [71, 72], and its downstream targets including NFAT, NFκB and MAPK are involved in the pathogenesis of T-cell malignancies [73]. Targeting the ITK-dependent IL-2 receptor (CD25) signaling pathway in T- cell lymphomas / leukemias with anti-CD25 monoclonal antibodies has shown promising efficiency [74]. These results indicate that ITK is a potential therapeutic target for T cell malignancies. Recently, a chromosomal translocation between Itk and spleen tyrosine kinase (Syk) leading to T-cell lymphoma was identified [75-77]. This translocation fuses the PH and TH domain from ITK to the SYK kinase

domain, resulting in activated SYK. The activation of ITK-SYK fusion protein is dependent on PI3k signaling [78], suggesting that PI3K inhibitors may be effective in treating ITK-SYK initiated T-cell lymphoma [79, 80].

ITK inhibitors

Numerous lines of evidence suggest ITK as a potential therapeutic target in various diseases and demonstrate the importance of developing small molecule inhibitors of ITK. With the exception of Ibrutinib, the first FDA approved ITK inhibitor; the majority of small molecule ITK inhibitors are still in the early stages of development

Aminothiazole based ITK inhibitors

By screening compound libraries and analyzing structure and activity relationships (SAR), two aminothiazole based molecule inhibitors of ITK, BMS-488516 and BMS-509744, were identified [81-83]. They are ATP competitive inhibitors, indicating their binding to the ATP binding site of the ITK kinase domain. These two ITK inhibitors had more than 200-fold selectivity versus other Tec family tyrosine kinases and potently inhibited ITK with half maximal inhibitory concentration **(**IC_{50}) values of 96 nM and 19 nM, respectively. Both compounds inhibited TCR-induced PLCγ1 phosphorylation, T-cell proliferation, calcium mobilization and IL-2 production. BMS-509744 efficiently suppressed lung inflammation in a mouse model of allergic asthma.

Benzimidazole based ITK inhibitors

The benzimidazole series of compound 1 was identified by high throughput screening [84]. It is an ATP competitive and has an IC_{50} value of 12 nM in the ITK enzyme assay. Further modifications improved the selectivity, potency and drug-like properties including stability and oral administration. Compound 10n and 10o inhibited IL-2 production with an IC_{50} of 240 nM and 690 nM, respectively, in a human whole blood assay. Oral administration of compound 10n inhibited the production of IL-2 and IL-4 in a mouse model of T cell activation induced by anti-CD3 [85].

Aminopyrimidine based ITK inhibitors

Compound 44 is a potent small molecule inhibitor of ITK identified by high-throughput screening of a library containing 468,462 compounds [58]. It has an IC_{50} of 65 nM in inhibiting ITK kinase activity. Compound 44 inhibited the secretion of IL-2 and IFN-γ and the proliferation of activated T cells. In two models of inflammatory skin diseases, compound 44 significantly reduced skin inflammation.

6.4 3-aminopyride-2-ones based ITK inhibitors

This is a new series of ITK inhibitors identified by structure-based design, starting from 3-aminopyride-2-ones, a fragment designed de novo [86]. Among various derivatives, the compound 7v represented the best ITK inhibitor with good potency and selectivity.

Indolylndazole based ITK inhibitors

ITK inhibitor 11o was identified based on indolylndazole libraries [87]. It had enzymatic activity at 11 nM and cellular activity at 20 nM. In an anti-CD3-induced IL-2 mouse model, intravenous or oral administration of 11o (10mg/kg) inhibited IL-2 secretion, and this drug was well tolerated without obvious side effects.

CTA056

CTA056, 7-benzyl-1-(3-(piperidin-1-yl)propyl)-2-(4-(pyridin-4-yl)phenyl)-1*H*-imidazo[4,5-*g*]quinoxalin-6(5*H*)-one, was developed through screening a library comprising 9600 compounds, followed by molecular modeling and analysis of SAR [88]. It showed the highest inhibition toward ITK with an IC_{50} of 100 nM, followed by BTK with an IC_{50} of 400 nM. CTA056 treatment in T cells inhibited the phosphorylation of ITK and its downstream targets including PLCγ1 and reduced the secretion of IL-2 and IFN-γ. CTA056 selectively targeted malignant T cells expressing ITK, including acute lymphoblastic T-cell leukemia and cutaneous T-cell lymphoma. In a xenograft model of T cell leukemia, CTA056 treatment (5 mg/kg, twice a week, intratumoral injection) prevented tumor growth.

6.7 ITK inhibitors targeting cysteine-442 in the ATP pocket

Ibrutinib is an irreversible inhibitor of BTK and ITK [89, 90]. It binds to cysteine-481 residue in BTK or cysteine-442 residue in ITK and inhibits downstream activation of BCR or TCR, respectively. After TCR stimulation in primary $CD4^+$ T cells and Jurkat T cells, Ibrutinib inhibited activation of PLCγ1, NFAT, JunB and IKBα which are ITK downstream targets [89]. Interestingly, ibrutinib specifically inhibited Th2 T cell activation and Th2-type cytokine release and provided a selective advantage to Th1 and $CD8^+$ T cells which express RLK beside ITK. The inhibitory effect of ibrunitib on ITK was further validated in primary CLL samples and several murine models of CLL, parasitic infection (Leishmania major) and infectious disease (Listeria monocytogenes) [89]. Ibrutinib has shown significant clinical activity in the treatment of mantel cell lymphoma (MCL) and CLL [91-93], and been approved by the U.S. Food and Drug Administration (FDA) for the treatment of these two malignancies.

Compound 12 is another irreversible ITK inhibitor targeting Cysteine-442 in the ATP pocket [94]. In activated human peripheral blood mononucleated cells (PBMCs), compound 12 had the most potency at inhibiting IL-12, followed by IFNγ, IL-13 and IL-17. The inhibition of T cell activation by compound 12 was demonstrated in a rat model by inhaled delivery of this drug. The inhibitory effect of compound 12 on Th1, Th2 and Th17 T cells suggests that it might also inhibit RLK, which needs verification by further study.

Other ITK inhibitors

ITK inhibitors based on pyrazolyl-indole [95] or thienopyrazole [33] were described in previous studies. Rosmarinic acid, a natural compound, was also shown to inhibit activation of ITK, PLCγ1, NFAT and Ca^{2+} mobilization [35]. Instead of targeting the ATP site in ITK, inhibitors targeting ITK allosteric sites were discovered and characterized [96].

Conclusion

Intensive studies have demonstrated the essential role of ITK in T cell development and differentiation, implicating ITK as a potent therapeutic target in various diseases including Th2 cell related immunodeficiency and inflammation. The success of ibrutinib, a BTK/ITK-targeting inhibitor, in the treatment of B-cell leukemia and lymphoma encourages the development of such targeted therapy in T-cell related diseases. Though a number of ITK inhibitors have been discovered, most of them are still in their early stages of development and more effort is needed before their application in clinic. In addition, due to a compensatory role of RLK to ITK in Th1 T cells, development of dual inhibitors targeting both ITK and RLK will be important.

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