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Review

Exploration of the therapeutic aspects of Lck: A kinase target in inflammatory mediated pathological conditions

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ABSTRACT

Lck, a non-receptor src family kinase, plays a vital role in various cellular processes such as cell cycle control, cell adhesion, motility, proliferation and differentiation. As a 56 KDa protein, Lck phosphorylates tyrosine residues of various proteins such as ZAP-70, ITK and protein kinase C. The structure of Lck is comprised of three domains, one SH3 in tandem with a SH2 domain at the amino terminal and the kinase domain at the carboxy terminal. Physiologically, Lck is involved in the development, function and differentiation of T-cells. Additionally, Lck regulates neurite outgrowth and maintains long-term synaptic plasticity in neurons. Given a major role of Lck in cytokine production and T cell signaling, alteration in expression and activity of Lck may result in various diseased conditions like cancer, asthma, diabetes, rheumatoid arthritis, psoriasis, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, atherosclerosis etc. This article provides evidence and information establishing Lck as one of the therapeutic targets in various inflammation mediated pathophysiological conditions.

1. Introduction

Inflammation forms physiological response towards any unwanted external and internal intrusion by pathogens, damaged cells, irritants, etc [1]. In recent years, several research groups are working on different mechanisms involved in the inflammatory responses and pathological conditions arising afterwards [2]. Biopharmaceuticals based anti-inflammatory therapies involving monoclonal anti-bodies have been found very efficient and safe, but their development is very expensive [3]. Thus, the current research mainly focus on investigation of novel small molecule heterocycles as therapeutic agents for inflammatory disorders. Several novel molecular targets such as kinases have now been established as key mediators in multiple pathological conditions. The tyrosine kinases are involved in various cellular processes [4] especially inflammation [5]. There are three main sub-classes of kinases, Src, Tec and Syk kinases, that are intimately implicated in TCR (T cell antigen receptors) signaling, a foremost step in cellular recognition of attacking pathogens and tissue damage [6]. The over-activation of these tyrosine kinases causes a change in gene expression of the affected cells. The most affected genes are of cytokines that coordinate the duration as well as extent of inflammation. The tyrosine kinases are also involved in functioning and signaling of various inflammatory cytokines such as TNF-α, IL-2 and IL-6 [7]. Out of these, Lck, an important but sidelined Src kinase, is known to be a critical component and is well studied for several inflammatory mediated pathological conditions. This review discuss the rationality of targeting Lck kinase for the management of different inflammatory mediated pathological conditions.

2. Lck

Lck, also known as lymphocyte-specific protein tyrosine kinase [8], is a member of Src family of non-receptor protein tyrosine kinases. It is a 56 KDa protein which phosphorylates a number of proteins like ZAP-70, ITK, protein kinase C, and PI3K, and thereby regulates several cellular processes including cell cycle control, cell adhesion, cell motility, cell proliferation and cell differentiation. Lck is expressed in T cells, NK cells and brain [9]. *Lck* gene is located on short arm of chromosome 1 at position of 34.3 and is composed of a segment with exon count 14 [10]. Mislocalisation of lck can impair thymocyte differentiation and may lead to the formation of thymomas [11]. Lck is involved in the phosphorylation of intracellular signaling molecules at the tyrosine residue, thereby regulating lymphocytes. This kinase has been reported to be associated with cytoplasmic domains of CD4 and CD8 along with the beta chain of IL-2 receptor and is involved in the steps of TCR mediated T cell activation. Studies involving Human Jurkat cell line have disclosed that the suppression of Lck expression results in inhibition of TCR signaling. Additionally, studies have

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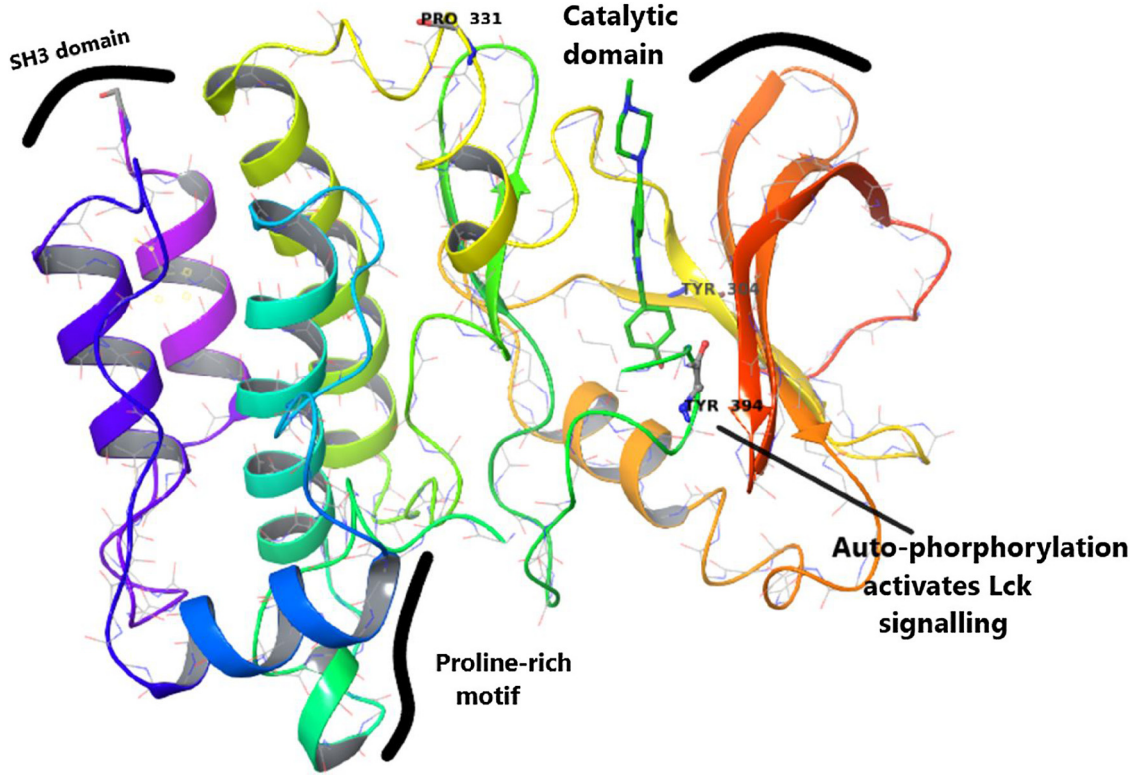

Fig. 1. Structure of various domains of Lck.

disclosed that the targeted disruption of *lck* results in early arrest of thymocyte maturation. This block occurs at a developmental arrest point that is similar to that seen in the TCR β–RAG, and CD3ε-deficient mice [12]. Such studies have established the key role of Lck in TCR signaling pathway. Since Lck is involved in T cell proliferation and differentiation, therefore, new small molecules with Lck inhibitory activity can be of great relevance to treat T cell mediated diseases [13].

2.1. Structure of Lck

As mentioned previously, Lck consist of three domains, one SH3 in tandem with a SH2 domain at the amino terminal and the kinase domain at the carboxy terminal [14]. The C-terminal lobe contains an alpha-helical activation loop which forms the site of phosphorylation [15]. Both SH2 and SH3 domains are small and folded modules involved in protein-protein interactions, while SH2 regulates interactions with phosphotyrosine containing elements, SH3 regulates interactions with proline rich elements in signal transduction pathways. The SH3 domain has a beta-barrel architecture consisting of n-Src and RT loops as shown in Fig. 1 [16]. These loops lie at either end of a hydrophobic surface which form a recognition site for proline-rich motifs. SH2 domain consists of a central beta-sheet and the loops which connect them to form two pockets. First pocket coordinates with phosphotyrosine while second pocket binds with hydrophobic residues that lie towards C-terminal from phosphotyrosine. The phosphotyrosyl recognition pocket is highly conserved among SH2 domains, and contains an arginine residue that forms requisite electrostatic interactions with the phosphorylated tyrosine [17]. The N-terminal contains five beta-strands and a single alpha-helix, known as the C helix. The kinase domain is a highly conserved structure and contains the catalytic function of lck. Activation of Lck is reported to be regulated via site specific dephosphorylation at Tyr 505 by the activating phosphatase CD45 [18]. Although studies have already disclosed that resting T cells are already dephosphorylated at Tyr 505 and therefore occur in partial active state. Conversely, autophosphorylation of Tyr 394 has been claimed to be involved in complete Lck activation. As shown in Fig. 1, Tyr 394 is found deep buried in the catalytic domain of Lck. Mutagenesis studies have suggested that phosphorylation of Tyr 394 is required for optimal function of Lck [19]. Further, in 2017, a study by Courtney et al disclosed an unappreciated role for a phosphosite, Tyr192, within the SH2 domain of Lck that profoundly affects the amount of active Lck in cells. Their study identified that modification of Tyr192 impairs the ability of CD45 to associate with Lck in cells and de-phosphorylate the C-terminal tail of Lck, which in turn prevents its adoption of an active open conformation. Overall these results suggest a negative feedback loop which works similar to signaling events that regulate active Lck amounts and TCR sensitivity [20]. Thus, catalytic domain regulates various cellular processes like metabolism, transcription, cell cycle, cell movement, differentiation and apoptosis. The unique N-terminal mediates interaction with T cell surface co-receptors CD_4 and CD_8 by zinc coordination with conserved cysteine motifs present in both proteins [21]. Interestingly, Lck serves as an example where alteration in the site of phosphorylation results in alteration of physiological response [22]. Phosphorylation in Lck occurs at serine 42 and 59 which is carried out by protein kinase C, eventually altering the function and catalytic activity of the kinase (Fig. 1) [23].

2.2. Physiological role of lck

At physiological level, Lck primarily plays a key role in the selection and maturation of developing T cells in the thymus and in the function of mature T cells. It is also involved in the proper activation of TCR-linked signal transduction pathways [24]. The earliest steps involved in the initiation of TCR signaling involve recruitment of a CD4 or CD8 coreceptor-associated kinase and Lck. Followed by phosphorylation of the tyrosine residues in the cytoplasmic ζ and CD3 chain ITAMs (immunoreceptor tyrosine-based activation motifs). Lck then phosphorylate tyrosines 315 and 319 on Zap70, followed by phosphorylation of Tyr 493 present on the activation loop in order to change its conformation from active to inactive [25]. Binding of Lck to tyrosine 319