Deregulation of IL-4/IL-13-induced STAT6 signaling in viral oncogenesis

Chong Wang¹, Fang Wei², Qiliang Cai¹

¹MOE & MOH Key Laboratory of Medical Molecular Virology, School of Basic Medicine, Shanghai Medical College, Fudan University, Shanghai 200032, P. R. China
²ShengYushou Center of Cell Biology and Immunology, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

Correspondence: Qiliang Cai
E-mail: qiliang@fudan.edu.cn
Received: November 22, 2015
Published online: January 11, 2016

Signal transducer and activator of transcription (STAT)-6 is previously shown to involve in adaptive immunity by transducing signals from extracellular cytokines IL-4 and IL-13. Emerging evidences have demonstrated that STAT6 signaling is also employed for anti-tumor growth in cancers and innate immunity in response to virus infection. However, in the past decade, it has become a hot spot in the field to address how STAT6 signaling is selectively manipulated by viruses for achieving a favorable cellular environment during infection. In this review, we summarize recent progress about how STAT6 signaling is targeted by virus infection and contributes to viral oncogenesis, and highlights the potential therapeutic strategy for disrupting STAT6 signaling in viral cancers.

Keywords: STAT6; Virus; Cancer


Copyright: © 2015 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Introduction

Signal transducers and activators of transcription (STAT) is a family of transcription factors which mediate signaling transduction in response to growth factors, chemokines, hormones and cytokines [1-3]. STAT6, as one member of STAT family, shares a similar domain organization as other STATs which consist of a central DNA-binding domain (DBD, which binds to specific DNA sequence), a conserved SH2 domain and a transactivation domain (TAD) at carboxyl terminus (Figure 1). Classically, STAT6 is activated by cytokine IL-4 or IL-13 binding to their cognate receptors, which induce the recruitment and activation of Janus tyrosine kinases (JAKs) [4, 5]. Activated JAKs then phosphorylate IL-4 or IL-13 receptor on tyrosine residues which provide a docking site for cytoplasmic STAT6. Once STAT6 binding to the receptor, STAT6 itself is phosphorylated on tyrosine 641 (Y641). The phosphorylated STAT6 then dimerizes through its SH2 domain and translocates into nucleus, where activates STAT6 binding to a highly conserved DNA sequence motif (TTCN3GAA) within downstream gene promoter, and in turn regulates gene expression [6, 7]. It has been demonstrated that IL-4 and IL-13 share IL-4Rα/IL-13Rα1 receptor, although they could recognize
Deregulation of STAT6 signaling associates with many cancers

<table>
<thead>
<tr>
<th>Virus</th>
<th>Target gene</th>
<th>STAT6 Status</th>
<th>Function</th>
<th>Related Cancer</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>IL-4</td>
<td>Activation, mutation</td>
<td>Pathogenesis, immune evasion</td>
<td>Follicular lymphoma</td>
<td>[29-32]</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>Activation</td>
<td>Cell proliferation and survival</td>
<td>B-cell acute lymphoblastic leukemia (B-ALL)</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>Activation</td>
<td>Inhibit apoptosis</td>
<td>Chronic lymphocytic leukemia (CLL)</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>JAK2, SOCS1</td>
<td>Activation</td>
<td>ND</td>
<td>Primary mediastinal B-cell lymphoma (PMBL)</td>
<td>[35-37]</td>
</tr>
<tr>
<td></td>
<td>IL-7, IL-15</td>
<td>Activation</td>
<td>ND</td>
<td>Cutaneous T cell lymphoma (CTCL)</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>SOCS1, SHP-1</td>
<td>Activation</td>
<td>Resistance to apoptosis</td>
<td>Colon cancer</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>SOCS1, JAK2</td>
<td>Activation</td>
<td>ND</td>
<td>Lymphocyte- predominant Hodgkin lymphoma</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>Activation</td>
<td>ND</td>
<td>Prostate cancer</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>IL-4, JAK1</td>
<td>Activation</td>
<td>Cell proliferation and survival</td>
<td>Thyroid Cancer</td>
<td>[41]</td>
</tr>
<tr>
<td>KSHV</td>
<td>IL-4, IL-13, SHP1, JAK1/2</td>
<td>Activation</td>
<td>Cell proliferation and survival, KSHV latency</td>
<td>Primary effusion lymphoma</td>
<td>[22,23]</td>
</tr>
<tr>
<td>EBV</td>
<td>IL-13</td>
<td>Activation</td>
<td>Cell proliferation and survival</td>
<td>classical HL (HRS cells)</td>
<td>[14]</td>
</tr>
</tbody>
</table>

Note: ND, not determined.

Figure 1. Schematic of protein structure of STAT6 and its interacting partners. STAT6 protein consists of approximately 840 amino acids, each domain from left to right is presented and highlighted: α-helices (region rich in α-helices), DBD (DNA binding domain), SH2 (src homology 2 domain), TAD (transactivation domain). Phosphorylation site of STAT6 was shown on tyrosine (Y) or serine (S); STAT6 65 kD, the proteolytic isofrom found primarily in mast cells and T cells. Representative proteins associated with STAT6 specific domain including dimerization were shown at the bottom panels.

On their own unique receptor IL-4Rα/IL-2Ryc and IL-13Rα2, respectively. The details about the STAT6 upstream negative regulators including SHP1/2 and SOCS1/3, and downstream target genes could partially refer to Figure 2 and previous review [5].

Studies in STAT6 knockout mice model have shown that deficient of STAT6 results in functional loss of positive signaling induced by IL-4 and IL-13, including diminished expression of several B cell surface makers like MHC class II, CD23, and IL-4R. Furthermore, in B lymphocytes cells, deficiency of STAT6 causes the disability to produce IgE following immunization with anti-IgD in vivo [8-10], and STAT6-deficient T lymphocytes also fail to differentiate into Th2 cells in response to either IL-4 or IL-13 [11]. On the other hand, STAT6 also mediates IL-4-inhibition of both Th1 type T-cell differentiation and IFN-γ induced gene expression in macrophage [12,13].

STAT6 is constitutively activated in many cancers

In addition to the role of STAT6 in regulating cell differentiation and immune response, emerging evidences have shown that STAT6 also displays a pivotal role in regulating cell apoptosis and tumor progress (Table 1). Notably, constitutive activation of STAT6 is the most common in many cancers. For example, In Hodgkin and Reed-Sternberg (HRS) cells of Hodgkin lymphoma, a high incidence with 25 of 32 examined HRS cells showed positive for constitutive activation of STAT6, and further studies revealed that the autocrine of interleukin IL-13 by HRS cells is the key factor for the activation of STAT6 [14]. Similarly, constitutive activation of STAT6 is also found in primary mediastinal large B-cell lymphoma, although this STAT6 activation is not due to an autocrine of IL-13 secretion, but rely on activation or overexpression of the Janus kinase (JAK) 2 and deficiency of SOCS1 expression [15, 16]. Interestingly, it is also found that STAT6 constitutively activates in both primary prostate and colon cancer tissues [17, 18]. Knockdown of STAT6 in colon cancer cells leads to dramatically increases cell apoptosis [18], further supporting the notion that STAT6 plays an important role in tumor growth. However, how STAT6 is constitutively activated and contributes to oncogenesis remains largely unclear, albeit IL-13 is recently assumed to play a role in anti-tumor immunity and tumor growth [19].

STAT6 signaling is selectively manipulated for viral oncogenesis
is phosphorylated on both Ser$^{407}$ by TBK1 and Tyr$^{641}$, followed by dimerization and activation for immune cell homing $^{[20]}$. Interestingly, it was also reported that Herpesvirus sainimiri (HVS), a T-lymphotropic monkey herpesvirus, encodes tyrosine kinase-interacting protein (TIP) to induce nuclear translocation of STAT6, and activation of STAT6-dependent transcription in Jurkat T-cells $^{[21]}$. Supporting to this notion, our recent studies also revealed that STAT6 is also constitutively phosphorylated to a certain extent in some of KSHV-positive primary effusion lymphoma (PEL) cell lines, and the molecular mechanism behind STAT6 activation in PEL is due to autocrine of IL-13, down-regulation of SHP1 and in turn activation of JAK1 caused by KSHV primary infection $^{[22]}$. In contrast to STAT6 activation, our earlier studies have also revealed that interleukin-4 (IL-4) -induced immune response of B-lymphocyte activation and cell proliferation is suppressed in the KSHV latently infected PEL cells, and LANA (Latency-Associated Nuclear Antigen) encoded by KSHV during latency is the one to respond for blocking, and in turn reduces phosphorylation of STAT6 on Tyr$^{641}$ and concomitantly its DNA binding ability $^{[23]}$. Taken together, this evidence indicates that cytokine IL-4 and IL-13-induced selectively STAT6 signaling is precisely regulated by virus during different stage of infection.

**STAT6 signaling contributes to co-infection of oncogenic virus**

Deregulation of STAT6 signaling not only influence host gene expression, but also conversely plays a role in regulation of viral gene expression, replication and reactivation. For instances, in the Hodgkin Lymphoma with EBV type II latency infection, exposure of IL-4 or IL-13 induces the expression of Latent Membrane Protein-1 (LMP1) in the absence of EBNA2 (known to drive LMP1 expression in EBV type III latency) $^{[24]}$. In the case of viral co-infection, Lu group showed that Human Immunodeficiency Virus type 1 (HIV-1) - encoded Tat protein could activate KSHV lytic replication which is partially dependent on IL-4/STAT6 signaling pathway $^{[25]}$, and our recent studies also showed that co-infection of EBV with KSHV is able to block the constitutive activation of STAT6 in PEL cells $^{[22]}$. Intriguingly, studies from Virgin group revealed that co-infection of MHV68 (a murine herpesvirus homologs of KSHV) with Helminth also leads to activation of IL-4/STAT6 signaling pathway, and in turn promotes viral reactivation by binding to the lytic master gene ORF50 promoter $^{[26]}$. Consistent with this conclusion, treatment of KSHV latently infected cell line BCBL1 with IL-4 induces KSHV life cycle switch from latency to lytic infection $^{[26]}$, which further supports our previous finding that LANA blocks IL-4 induction during KSHV latency $^{[23]}$. This

In regarding to the role of STAT6 signaling in the virus associated diseases, recent studies showed that STAT6 is also manipulated by viruses for antiviral innate immunity and induction of viral pathogenesis. For examples, when HeLa cells were primarily infected with Herpes simplex virus 1 (HSV-1) or Sendai virus (SeV), STING is activated and recruit STAT6 to endoplasmic reticulum (ER) where STAT6

---

**Figure 2. Overview of viral engagement with STAT6 signaling cascade.** STAT6 signaling is classically associated with stimulation of cytokine IL-4 or IL-13. When IL-4 and IL-13 binds to their cognate receptor IL-4Rα/IL-4Rγc and IL-4Rα/IL-13Rγ1, respectively, JAK kinase is activated, which in turn phosphorylates IL-4Rα on specific tyrosine residues. The phosphorylated tyrosine in the receptor chain thus provide docking sites for STAT6 monomers, which induce tyrosine phosphorylated themselves by the receptor-associated JAK kinases, phosphorylated STAT6 then dimerize and translocate into nucleus where the active STAT6 dimers bind to target DNA sequence and induce transcriptional activity. In the process of STAT6 activation, many cellular proteins are involved in the negative regulation of STAT6 signaling like SHP (protein tyrosine phosphatase) and SOCS (Suppressors of Cytokine Signaling) family. Functional loss of this negative regulator is commonly associated with many cancers and viral infection. In KSHV positive cells line, STAT6 is activated through inhibiting SHP1 and activation of JAK1/2. During HSV and SeV infection, STING is activated and in turn recruits STAT6 into ER where STAT6 is activated by TBK1. In addition, STAT6 can also regulate viral gene expression and replication as shown in the figure.
indicates that STAT6 signaling also contributes to viral co-infection.

**Future Perspective**

Due to STAT6 activation correlates with pleiotropic functional response induced by IL-4 and IL-13, selective activation of STAT6 signaling pathway in many cancers provides us a hint that STAT6 may be a potential target in the treatment of cancers. Indeed, knockdown of STAT6 by shRNA in human colon cancer cells increased cancer cell apoptotic rate than that in the control cells [18]. Also, in non-small cell lung cancer, overexpression of miR-361-5p (which specific target STAT6) significantly inhibits STAT6 expression and reduces tumor growth in a nude mouse xenograft model [27]. Moreover, knockdown of both BCL6 and STAT6 by siRNA increases the sensitivity of PBL cells to R-CHOP treatment [28]. For virus associated cancers, in the view of the roles of constitutively activated STAT6 in promoting KSHV associated PEL cell growth and survival, there is a possibility that knockdown of STAT6 may inhibit PEL progression. Meanwhile, recent studies demonstrated that STAT6 activation induced by IL-4 could reactivate KSHV lytic replication through binding to RTA promoter [29], indicating that reagents used to activate STAT6 pathway may be an appropriate strategy to reactivate KSHV from latency and promote the clearance of KSHV by the host cells, and may eventually decrease the incidence of cancers caused by KSHV infection.

**Conflicting interests**

The authors have declared that no conflict of interests exist.

**Acknowledgements**

The authors would like to apologize to the many researchers who have contributed to this area of research but have not been cited in this review due to space limitations. This work is supported by the Research and Innovation Program of the Shanghai Municipal Education (13zz011), the National Natural Science Foundation of China (81471930, 81402542), and the National Key Basic Research “973” program of China (2012CB519001). FW is a scholar of Pujiang Talents in Shanghai. QC is a scholar of New Century Excellent Talents in University of China.

**Author contributions**

C.W. and Q.C. conceived and wrote the manuscript, F.W. analysed the results.

**References**


