

Establishing the role of tyrosine kinase 2 in cancer

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Keywords: cancer, cytokines, JAK-STAT signalling, lung cancer, tumor therapy, TYK2

Abbreviations: EGF, epidermal growth factor; FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; JAK, Janus kinase; NSCLC, non-small cell lung carcinoma; STAT, signal transducer and activator of transcription; TYK, tyrosine kinase

Tyrosine kinase 2 (TYK2) is a member of the Janus family of non-receptor tyrosine kinases involved in cytokine signaling. TYK2 deficiency is associated with increased susceptibility to mycobacterial and viral infections, hyper IgE syndrome as well as with allergic asthma. However the precise role of TYK2 in oncogenesis and tumor progression is not clear yet. Tyk2-deficient mice are prone to develop tumors because they lack efficient cytotoxic CD8⁺ T-cell antitumor responses as a result of deficient Type I interferon signaling. However, as TYK2 functions downstream of growth factor receptors that are often hyperactivated in cancer, inhibiting TYK2 might also have beneficial effects for cancer treatment.

Introduction

Recent research on the inhibition of Janus kinases (JAKs) has highlighted the importance of these molecules in the development and therapy of several diseases including cancer. However, most of the work published so far deals with JAK2 or JAK3. We therefore sought to analyze the role of another JAK, tyrosine kinase 2 (TYK2), in disease, notably cancer, in order to find out possible strategies for the development of new therapeutic approaches. TYK2 is a ubiquitously expressed non-receptor protein tyrosine kinase. TYK2 belongs to the subfamily of JAKs that transduce cytokine-derived signals in immune and hematopoietic cells. JAKs are important for cellular growth as well as for the development and differentiation of various cell types, and are normally associated with cytokine receptors, especially those for Type I and Type II cytokines. Thus, JAKs most often respond to hematopoietic cytokines and growth factors (Table 1). TYK2 is associated to five different cytokine receptors, i.e., the interferon α (IFN α) receptor 1 (IFNAR1), the interleukin (IL)-12 receptor β 2 (IL-12R β 2), the IL-10 receptor β (IL-10R β), the IL-6 receptor α (IL-6R α) and the IL-13 receptor α (IL-13R α) (Fig. 1).^{1,2} TYK2 plays a diverse role in cytokine signal transduction (Fig. 1; Table 1). In particular, TYK2 is never solely responsible for cytokine signaling, but

rather collaborates with JAK1 and JAK2, but not with JAK3.³ Its contribution to signaling is not yet clearly described for all of the abovementioned cytokines, and notably for cytokines of the IL-6 family that use the gp130 receptor. Moreover, it has been found that the role of the JAKs is species-dependent. For example, the relevance of TYK2 in IL-6, IL-12 and IFN α / β signaling is different between mice and humans. Indeed, while IL-6 signaling is not functional in human patients bearing TYK2 defects, it is perfectly normal in Tyk2-deficient mice.⁴⁻⁶ Immune responses are based on a functional JAK-dependent signal transduction. If signaling through one JAK is interrupted, severe pathological outcomes can ensue (e.g., cancer and immunodeficiencies),^{1,2} as we will discuss in this review with a focus on the role of TYK2 in human diseases, especially cancer.

JAK-STAT Signal Transduction

The JAK family comprises four kinases, JAK1, JAK2, JAK3 and TYK2. Since they are highly homologous to each other in structure as well as in function they are considered as isozymes.³ All JAKs consist of seven JAK homology (JH) domains (JH1-JH7) and are rather large proteins, with molecular weights ranging from 120 to 130 kDa (Fig. 2A).³ The catalytic domain (JH1) is located at the C-terminus of the molecule, followed by the JH2 domain, which is also known as pseudokinase domain. This domain is characteristic of JAKs: it does not have catalytic activities but regulates phosphorylation. JH3 and JH4 form a SRC-homology 2 (SH2) domain-like structure whose function is not yet fully understood. The so called "FERM" region, which is associated with the intracellular tails of cytokine receptors, consists of the JH5, JH6 and JH7 domains.^{1,7}

The signal transducers and activators of transcription (STAT) family of transcription factors comprises seven members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6), all of which are activated by cytokines (Table 1). STATs consist of six domains. An N-terminal domain is followed by a coiled-coil domain and a DNA binding domain (DBD). Then, after a linker domain, the SH2 and transactivation domain (TAD) can be found, which are important for protein phosphorylation (Fig. 2B).^{8,9}

Cytokine-driven signal transduction starts when the cytokine receptor is stimulated by the binding of a specific ligand. In this context, JAKs are activated by autophosphorylation (Fig. 2C) and become able to phosphorylate the intracellular tail of the receptor,

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Submitted: 09/28/12; Accepted: 11/09/12
<http://dx.doi.org/10.4161/onci.22840>
Citation: Übel C, Mousset S, Trufa D, Sirbu H, Finotto S. Establishing the role of tyrosine kinase 2 in cancer. Oncoimmunology 2013; 2:e22840

Table 1. JAK-STAT-dependent cytokine signaling

Cytokine	JAK
Common γ -chain cytokine family, ⁷⁷ IL-10 family, ^{2,77} IL-6, ⁷⁷ IL-13, ² IL-27, ² IFN α / β / γ , ^{2,77} G-CSF, ^{78,79} OSM, ^{2,77} LIF, ^{2,77} CNTF ²	JAK1
Common β -chain cytokine family, ⁷⁹ IL-12 family, ² IL-6, ^{2,77} IL-11, ^{2,77} IFN γ , ⁷⁷ G-CSF, ⁷⁷ GH, ^{77,79} EPO, ^{77,79} OSM, ^{2,77} LIF, ^{2,77} CNTF, ² Prl, ^{77,79} LIF, ⁷⁷ TPO ⁷⁹	JAK2
Common γ -chain cytokine family ⁷⁷	JAK3
IL-10 family, ^{2,6,77} IL-12 family, ^{2,6} IL-6, ^{2,6,77} IL-11, ^{2,77} IL-13, ² IFN α / β , ^{2,6,77} G-CSF, ⁷⁸ OSM, ^{2,77} LIF, ^{2,77} CNTF ²	Tyk2
Cytokine	STAT
IL-10 family, ⁸⁰ IL-6, ⁷⁹ IL-11, ⁷⁹ IFN α / β / γ , ⁷⁹ OSM, ⁷⁹ LIF, ⁷⁹ CNTF ⁷⁹	STAT1
IL-28, ⁸⁰ IL-29, ⁸⁰ IFN α / β ⁷⁹	STAT2
Common γ -chain cytokine family, ⁷⁹ IL-10 family, ⁷⁹ IL-6, ⁷⁹ IL-11, ⁷⁹ IFN γ , ⁷⁹ G-CSF, ⁷⁹ OSM, ⁷⁹ LIF, ⁷⁹ CNTF ⁷⁹	STAT3
IL-12, ⁷⁹ IL-23, ⁷⁹ IFN α / β ⁷⁹	STAT4
Common γ -chain cytokine family (without IL-4), ⁷⁹ IL-10 family, ⁸⁰ Common β -chain cytokine family, ⁷⁹ IFN γ , ⁷⁹ EPO, ⁷⁹ TPO, ⁷⁹ TSLP, ⁷⁹ GH, ⁷⁹ Prl ⁷⁹	STAT5a
Common γ -chain cytokine family (without IL-4), ⁷⁹ IL-10 family, ⁸⁰ Common β -chain cytokine family, ⁷⁹ IFN γ , ⁷⁹ EPO, ⁷⁹ TPO, ⁷⁹ TSLP, ⁷⁹ GH, ⁷⁹ Prl ⁷⁹	STAT5b
IL-4, ⁷⁹ IL-13 ⁷⁹	STAT6
Cytokine	Family
IL-2, IL-4, IL-7, IL-9, IL-11, IL-15, IL-21	Common γ -chain cytokines
IL-3, IL-5, GM-CSF	Common β -chain cytokines
IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29	IL-10
IL-12, IL-23, IL-27, IL-35	IL-12

The table describes the cytokines that use a certain Janus kinase (JAK) (upper part) in signal transduction that leads to the activation of certain STATs (lower part). Below members of different cytokine family members are listed.

enabling the binding of transcription factors of the STAT family (Fig. 2C).⁹ Upon phosphorylation, STATs dissociate from the receptor complex and form either homo- or heterodimers. Dimeric STATs acquire the ability to translocate to the nucleus and regulate gene expression.^{1,9} The activity of JAKs is negatively regulated by several suppressor of cytokine signaling (SOCS) proteins (SOCS1-SOCS7) as well as by cytokine inducible SH2-domain (CIS) proteins, the latter bind to cytokine receptors and block the recruitment and phosphorylation of STATs. Both SOCS and CIS proteins possess a SOCS box at their C-terminus, which functions as an E3 ubiquitin ligase and stimulates protein degradation via the proteasome. SOCS proteins also contain a kinase-inhibitory region (KIR), which can be used as a pseudosubstrate by JAKs, leading to their inactivation. In addition, tyrosine phosphatases like SHP-1, PTP-1B and CD45 regulate cytokine receptor and JAK phosphorylation. STATs can be also sumoylated by protein inhibitor of activated STAT (PIAS) molecules, leading to their degradation. Finally, nuclear STATs are generally dephosphorylated by specific phosphatases, leading to the inactivation of their transcriptional functions (Fig. 2D).^{1,10}

Inflammatory Diseases and Cancer

As chronic inflammation is a risk factor for the development of some types of cancer, we will first discuss the role of TYK2 in inflammatory diseases. TYK2 has been first described in 1992 as a component in the signal transduction pathway elicited by IFN α

and IFN β .¹¹ Since then, the role of TYK2 in cytokine signaling has been characterized in more detail. Most cytokines signaling via TYK2 are essential for the proper functioning of the immune system. Thus, if TYK2-dependent signal transduction is disrupted, a severe impairment of the immune system ensues. Minegishi et al. described a patient with a non-functional TYK2 protein, who had been diagnosed with many immunodeficiency conditions like the hyper IgE syndrome, atopic dermatitis, and an increased susceptibility to infections caused by viruses, fungi and mycobacteria. Analyses of peripheral blood cells showed a complete defect in IL-12 and IFN α / β signaling. IL-6, IL-10 and IL-23 signaling were also heavily compromised. Differentiation of naive CD4⁺ T lymphocytes was impaired, featuring reduced T_H1 polarization and IFN γ production. On the other hand, TYK2 deficiency led to an increase in T_H2 differentiation and T_H2 cytokine production. The clinical condition deriving from TYK2 deficiency can thus be classified as a primary immunodeficiency.¹² Another patient deficient in TYK2 has recently been reported to suffer from neurobrucellosis, Bacillus Calmette-Guérin and cutaneous herpes zoster infection, but not to show a hyper IgE Syndrome.¹³ Ishizaki et al. were able to demonstrate the importance of TYK2 in IL-12 and IL-23 signaling and to unveil the T-cell populations affected by this Tyk2 defect. Consistently, Tyk2-deficient mice suffer from inflammatory diseases, indicating a protective effect of Tyk2 in this setting.¹⁴

TYK2 is involved in the pathogenesis of other pathological conditions such as Alzheimer disease. It has been shown that the activation of TYK2 and STAT3 plays a crucial role in the accelerated

induction of neuronal cell death, which invariably accompanies this disease.¹⁵ Tyk2-deficient mice have been found to be resistant against autoimmune diseases like arthritis and experimental encephalomyelitis (EAE). Tyk2 signaling in T lymphocytes seems to be crucial for this protective effect.^{16,17} When single nucleotide polymorphisms were analyzed in Crohn's disease patients, TYK2 and STAT3 turned out to constitute biomarkers for the risk to develop this bowel inflammatory disease.¹⁸

IFN α and IFN β play an important role in the control of viral infections. In line with this notion, TYK2 is essential for the immune response to infections with vaccinia virus, lymphocytic choriomeningitis virus (LCMV) and murine cytomegalovirus (MCMV).^{4,19} IFN α and IFN β are also crucial in the response to endotoxins including lipopolysaccharide (LPS). High doses of LPS are able to trigger septic shock in wild-type, but not in Tyk2-deficient, mice. This is due to a significantly reduced production of IFN β by Tyk2-deficient macrophages.²⁰

A study had been conducted to investigate the role of TYK2 in leishmaniasis. TYK2 was shown to be essential for the proper cytotoxic functioning of natural killer (NK) cells and CD8⁺ T lymphocytes but not for T_H1 differentiation.²¹ Moreover, Tyk2-deficient mice exhibit an increased susceptibility to infections with opportunistic pathogens like *Toxoplasma gondii*, due to a compromised T_H1 differentiation.²²

Seto et al. have examined the role of TYK2 in the antigen-induced differentiation of helper T cells in a murine model of allergic asthma. In this setting, Tyk2-deficient mice exhibited increased allergic manifestations like T_H2-skewed differentiation pattern, elevated levels of IgE and IgG₁, and higher recruitment of eosinophilic granulocytes and CD4⁺ T cells as compared with wild-type mice. Altogether, these processes promote a strong inflammatory response after allergen confrontation. The number of goblet cells and the expression of Muc5ac, a mucin-encoding gene, were reduced in Tyk2-deficient mice as compared with wild type animals. Of note, the hyperresponsiveness of airways did not differ between these two mouse strains. Hence, TYK2 plays a differential role in modulating allergic asthma. On the one hand, it is important for regulating T_H2 differentiation and T_H2-induced antibody production. On the other hand, TYK2 is necessary for the induction of goblet cell hyperplasia in the airways.²³

Chronic inflammatory diseases are a risk factor for the development of some types of cancer. Indeed, it has been shown in several studies that chronic inflammation as well as autoimmunity are associated with the development of some neoplasms.

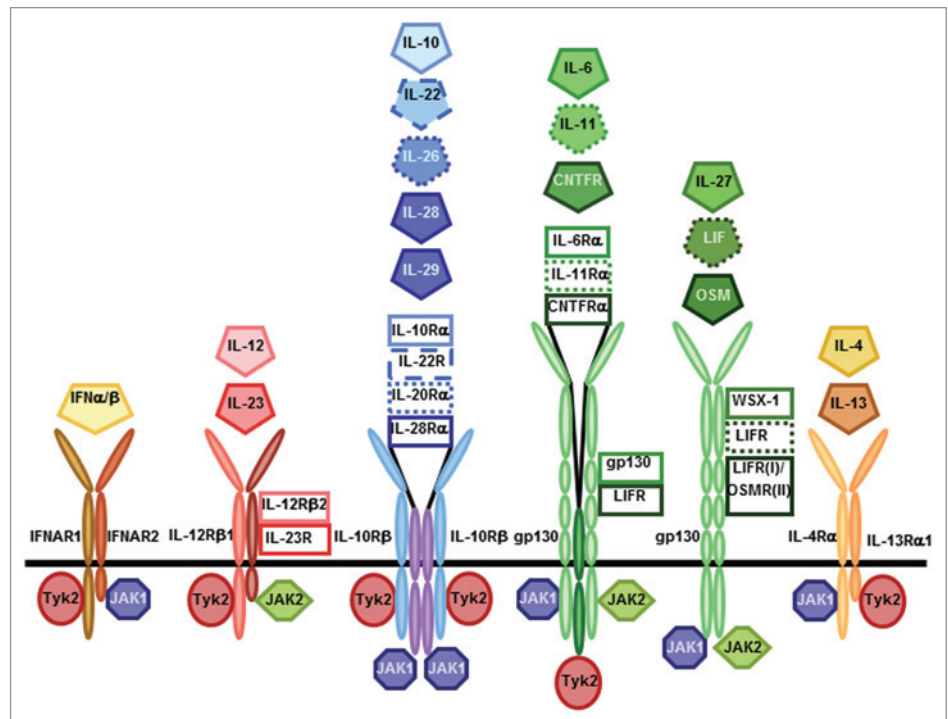


Figure 1. Overview of cytokine receptors and associated Janus kinases (JAKs). TYK2 is associated with several cytokine receptors, namely IFNAR1, IL-12R β 1, IL-10R β , IL-6R α , IL-11R α , CNTFR α and IL-13R α 1. IL-10, IL-22, IL-26, IL-28 and IL-29 share two receptor chains (IL-10R β) while the other two chains are similar for each cytokine, but different for members of this cytokine family. IL-6, IL-11, oncostatin M (OSM), leukemia inhibitory factor (LIF), IL-27 and ciliary neurotrophic factor (CNTF) share one receptor chain (gp130) while the two other chains are cytokine-specific. The importance of TYK2 varies in different cytokine signaling pathways. Its deficiency can be compensated to various extents depending on the respective receptor.

Therefore, patients suffering from conditions like celiac disease, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis are at an elevated risk of developing cancer. These patients should be monitored very carefully in order to detect the development of secondary diseases like cancer.^{30,31} This said, chronic asthma seems not to be associated with an increased incidence of hematological malignancies. On the contrary, multiple studies have revealed that allergic conditions appear to protect patients against the development of tumors by enhancing the ability of the immune system to prevent the growth of malignant cells. Although other studies show no associations between allergy and cancer development in general, an increased prevalence of cancer among asthmatic patients could only be detected for Hodgkin's and non-Hodgkin's lymphoma.³²⁻³⁴ Thus, the role of allergic asthma in cancer development remains to be fully elucidated, but the risk of developing cancer as a result of chronic asthma is certainly smaller than that stemming from most other inflammatory diseases.

Mast Cells and IgE in Tumors

Mast cells are important in the development of allergic diseases. In particular, they are crucial for establishing the inflammatory symptoms that are characteristic of this disease by releasing

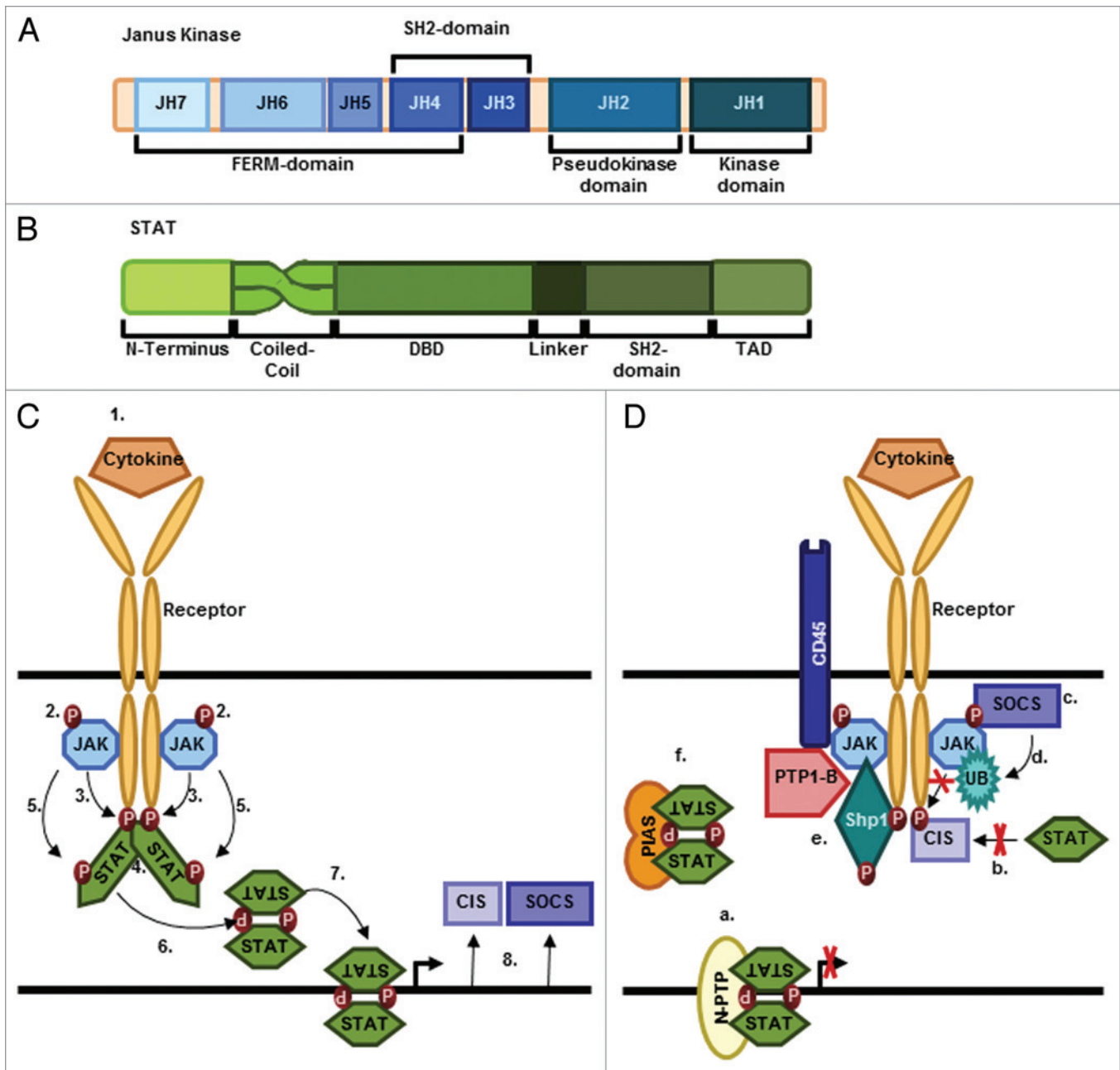


Figure 2. Positive and negative regulation of JAK-STAT signaling pathways. **(A)** Janus kinases (JAKs) consist of seven domains (JH1-JH7) exerting different molecular functions. **(B)** Signal transducer and activator of transcription (STAT) proteins consist of six different domains that mediate their interaction with cytokine receptors and - upon dimerization - with DNA. **(C)** Positive regulation of cytokine signaling via JAKs and STATs. Upon ligand binding, cytokine receptors dimerize (1) and undergoes a conformational change that allows for JAK autophosphorylation (2). JAKs then become able to phosphorylate the receptor chain at tyrosine residues (3). This recruits STAT proteins to the receptor chain (4), where they are phosphorylated by JAKs (5). Upon dimerization (6), phosphorylated STATs they translocate to the nucleus and regulate the expression of target genes by binding to the respective promoters (7). Examples of such targets include cytokine inducible SH2-domain (CIS)- and suppressor of cytokine signaling (SOCS)-coding genes (8). **(D)** Negative regulation of JAK-STAT signaling. The negative regulation of JAK-STAT signaling relies on different degradation pathways. Nuclear protein tyrosine phosphatases (N-PTPs) are able to remove the phosphate group from nuclear STATs, hence inhibiting signal transduction (a). This said, signal transduction also contributes to the negative regulation of KAK-STAT signaling by the upregulation of CIS and SOCS proteins, which inhibit STAT by binding to the receptor (b), by binding and hence inhibiting of JAKs (c) or by promoting the ubiquitination (Ub)-dependent degradation of JAKs (d). The phosphorylation of JAKs can be relieved by tyrosine phosphatases like CD45, SHP-1 and PTP1-B (e). The nuclear entry of STAT3 dimers can be inhibited by protein inhibitor of activated STATs (PIAS) molecules. Moreover, dimeric STAT can be directly dephosphorylated and degraded upon sumoylation (Su) (f). DBD, DNA-binding domain; SH2, SRC homology 2; SHP-1, SH2-containing phosphatase 1; TAD, transactivation domain.

pro-inflammatory mediators like histamine, cytokines and prostaglandins.²⁴ However, mast cells can also be found in the cancer microenvironment, where their role is not yet fully understood. On one hand, mast cells can induce angiogenesis and tissue remodeling, both of which facilitate tumor progression, by releasing cytokines the vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor β (TGF β), tumor necrosis factor α (TNF α) and IL-8. Enzymes released from mast cells like chymase, tryptase and metalloproteinases also promote neoangiogenesis and histamine is known to stimulate the proliferation of tumor cells. On the other hand, mast cells are able to inhibit tumor growth, to favor apoptosis and to decrease cell motility by secreting chymase, tryptase, TNF α , IL-1 and IL-6. The local concentration of these bioactive molecules is a critical determinant for its pro- or antitumor effects. It has been shown that intratumoral mast cells are able to induce tolerogenic dendritic cells (DCs) that suppress T-cell responses and thus promote tumor growth. In addition, it could be demonstrated that intratumoral mast cells are different from those found in the peri-tumoral zone with respect to their secretory profile.²⁵⁻²⁷

The role of IgE in cancer has not yet been analyzed in detail. Mast cells surrounding neoplastic lesions are commonly associated with a rather poor prognosis, but it is unknown whether this is influenced by the binding of IgE. It has been reported that IgE-driven antigen responses are associated with an efficient antigen cross-presentation and hence with an enhanced activity of CD8⁺ T cells. However, regulatory T cells might also be activated by this pathway.^{27,28} Antitumor IgE antibodies have been developed but all are currently being tested in preclinical settings only.^{27,29}

The Role of TYK2 in Cancer

The role of TYK2 and other JAKs in cancer has been extensively investigated. JAKs play important roles in the proliferation, differentiation, survival and apoptosis of normal as well as neoplastic cells.³⁵

TYK2 overexpression has been detected in several human breast cancer cell lines, as well as in prostate cancers and squamous cervical carcinomas.³⁶⁻³⁸ Several studies have been conducted in mice to analyze the role of TYK2 in cancer. In one of such studies, the function of TYK2 in an Abelson-induced tumor model was utilized. Infection with the Abelson murine leukemia virus leads to the development of B-cell lymphoid leukemia. In this setting, Tyk2-deficient mice are more prone to develop leukemia than wild type mice. Because tumor development in this model is relatively delayed, some mice are able to reject the neoplasm. Of note, while 30% of wild type mice were able to do so, all Tyk2-deficient animals succumbed to the disease. Additionally, Tyk2-deficient mice showed a shortened latency period, perhaps as a result of a heavily compromised tumor immunosurveillance and reduced cytotoxic activity of NK and NKT cells. TYK2 is thus especially important in both viral and non-viral lymphoid tumors.³⁹ Tyk2-deficient CD8⁺ T lymphocytes have been shown to lack cytotoxic activity and hence to be non-functional. IFN α/β signal transduction in CD8⁺ T cells is crucially important for the generation of a cytotoxic response. Since IFN α/β

signal transduction is disturbed in Tyk2-deficient mice, these animals lack CD8⁺ T-cell cytotoxicity and hence are unable to reject tumors.⁴⁰ The importance of IFN α/β signaling for CD8⁺ T-cell functions has also been shown with tumor-specific CD8⁺ T cells.⁴¹ A study on prostate cancer revealed that TYK2-mediated signal transduction facilitates the invasion of tumor cells into healthy tissues.³⁶ Zhang et al. have reported that mice lacking Tyk2 that are injected with breast cancer cells exhibit enhanced breast tumor growth and metastasis as compared with their wild-type counterparts. Thus, TYK2 deficiency alters the ability of the immune system to respond to tumors. In this model of breast cancer, myeloid-derived suppressor cells (MDSCs) seem to be more effective in Tyk2-deficient mice than in wild-type animals. In biopsic material from breast cancer patients TYK2 appeared to be downregulated, hence consistently influencing cell de-differentiation and the initiation of regional metastases.⁴² The function of CD4⁺ T cells, CD8⁺ T cells and NK cells was not altered in these mice. These results show that TYK2 plays an important role in suppressing the growth and metastatic potential of breast cancer. Thus, the screening of cancer, including breast cancer, patients in order to identify mutations in TYK2 might be useful for prognostic determination and for the identification of appropriate treatment modalities.⁴³ A distinct approach has been undertaken to analyze the role of TYK2 in oncogenesis. Thus, single nucleotide polymorphism (SNP) analyses showed that a polymorphism leading to a point mutation in the TYK2 kinase domain is associated with different solid tumors (breast cancer, colon cancer, gastric cancer) as well as with acute myeloid leukemia (AML).^{44,45}

Several studies have dealt with the role of TYK2 in B-lymphocyte development. IFN α is known to inhibit B-cell differentiation by inducing apoptosis. If TYK2 is missing, this regulatory mechanism is not working.⁴⁶ Thus, pro-B cells from Tyk2 deficient mice show a reduced phosphorylation of STAT3 and are protected from IFN β -dependent apoptosis.⁴⁷ Tyk2-deficient pro-B cells also have severe defects in mitochondrial respiration and ATP production.⁴⁸ Therefore, TYK2 plays an important role in regulation of B-cell apoptosis. Since TYK2 deficiency results in decreased B-cell apoptosis, this may facilitate the development of B-cell lymphomas.

In yet another study, functions of JAKs that support their consideration as new targets in therapies aimed at reducing chemoresistance have been unveiled. For example, TYK2 is phosphorylated downstream of the FGF2 receptor and is required for the full phosphorylation of extracellular signal-regulated kinase (ERK)1/2. The RNA interference (RNAi)-mediated silencing of JAK1, JAK2 or TYK2 inhibits FGF2-mediated proliferation and renders tumor cells more sensitive to chemotherapy-induced cell death. This happens independent of the canonical JAK-STAT-signal transduction pathway. TYK2 associates indeed with other kinases that are implicated in FGF2-mediated chemoresistance. Moreover, TYK2 is necessary for the induction of anti-apoptotic proteins like BCL-2 and MCL-1. This means that TYK2 and other JAKs are important modulators of FGF2-driven cell survival and that JAK inhibitors are likely to improve the efficacy of cancer therapies.⁴⁹

The role of TYK2 in oncogenesis, tumor progression and response to therapy is complex. While TYK2 overexpression has been detected in several cancer cell lines, Tyk2 deficiency in mice has been associated with an increased susceptibility to virus-induced tumors. In Tyk2-deficient mice, the CD8⁺ T-cell antitumor response appears to be defective, while MDSCs are highly active. Therefore, a comprehensive analysis of TYK2 expression in tumor cells is required for the precise understanding of its precise role in cancer. There are seemingly differences between cell lines, mouse models and human data.

JAK inhibitors. Several JAK inhibitors have been developed that may be applied in the clinical practice for cancer therapy.^{50,51} Some are designed to target the ATP-binding pocket, and hence to catalytically inactivate, JAKs.³ A JAK2 inhibitor (lestaurtinib, CEP701) is able to suppress STAT5 activation and hence the proliferation of primary erythroid cells from patients with myeloproliferative disorders like chronic myelogenous leukemia, polycythemia vera, essential thrombocythemia and chronic idiopathic myelofibrosis.⁵² Another JAK1/JAK2 inhibitor (ruxolitinib) is used for the experimental treatment of myeloproliferative neoplasms.⁵³ Recently, it has been shown that JAK-STAT signaling can get reactivated upon the prolonged exposure to a JAK2 inhibitor.⁵⁴ In one study, an RNAi approach was used to deplete TYK2 together with the tyrphostin A1, which blocks the activity of multiple protein tyrosine kinases. Although these experiments were performed *in vitro*, they showed that TYK2 inhibition may efficiently suppress the invasiveness of tumor cells.³⁶ It could also be demonstrated that the invasiveness of breast cancer cells can be blocked by TYK2 inhibition.⁵⁵ Thus, based on these results, the clinical use of a TYK2 inhibitor would mainly be appropriate in settings of Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus and some types of cancer. It is important to note that TYK2 deficiency is associated with a severely reduced tumor surveillance, resulting in increased tumor growth, as well as with a reduction in tumor cell invasiveness. Hence, the use of TYK2 inhibitors in cancer patients should be pondered very carefully.^{18,56,57}

This said, pan-JAK inhibitors are available that inhibit all JAKs, albeit at different concentrations.^{58–61} One of them, pyridone 6 (P6) has been shown to inhibit the growth of primary myeloma patient samples cultured in the presence of bone marrow stem cells.⁶¹ P6 has also been used in allergic inflammatory diseases like asthma and atopic dermatitis, successfully ameliorating clinical symptoms.^{58,59} STAT3 is mainly induced by IL-6 and IL-23, both of which signal via TYK2. The activation of STAT3 plays an important role in the pathogenesis of different diseases, especially cancer, as it often results from the activity of oncogenes like c-SRC, c-ABL, MET and ERBB2. In particular, a role for constitutively active STAT3 has been described in breast cancer.⁶² Skin squamous cell carcinomas that lack CD151 (tetraspanin) are responsive to treatment with reagents targeting TYK2, JAK2 and STAT3.⁶³ P6 has been demonstrated to inhibit STAT3 activation via TYK2 and JAK2 and hence to limit tumor growth.⁶⁴ Another pan-JAK inhibitor has been developed, yet its effects are predominantly due to the inhibition of JAK3, as its affinity for JAK3 is much higher than that for other JAKs. Of

note, a slightly modified variant of this inhibitor mainly JAK1 blocks activity.⁶⁰ Pan-JAK inhibitors normally lack structural specificity, which would make them comparatively more suitable for use in clinical approaches. The structural differences between the different JAK family members should be analyzed in more detail to allow for the development of highly selective inhibitors. In this sense, the analysis of a TYK2-inhibitor complex will be very important in discerning the functions of TYK2 from those of other JAKs.³

Lung cancer. Lung cancer is the most common type of cancer for both men and women and is associated with a high mortality rate. Each year, more people die of lung cancer than of breast, colon and prostate cancer combined.⁶⁵ The most common histological type of lung cancer (about 85% of all lung cancers) is non-small cell lung carcinoma (NSCLC), including squamous carcinoma, adeno-carcinoma and large cell carcinoma.⁶⁶ Surgery remains the therapy of choice for patients with early-stage (I and II) NSCLC.⁶⁷ Studies in patients with Stage IIIA NSCLC bearing ipsilateral mediastinal nodal metastases (N2) have shown the feasibility of resection after concurrent chemotherapy and radiotherapy, with promising rates of survival. Chemotherapy plus radiotherapy with or without resection are options for patients with Stage IIIA (N2) NSCLC.⁶⁸ For locally advanced, unresectable Stage IIIB or medically inoperable Stage IIIA NSCLC, the treatment includes platinum-based chemotherapy and thoracic radiotherapy. For patients with Stage IV disease and with a good performance status, platinum-based chemotherapy combined with vinorelbine, gemcitabine or taxanes is recommended.⁶⁹ In a large cohort (41,361 men and 30,804 women) of elderly NSCLC patients, the risks for short-term (up to 3 mo) and long-term (over 3 mo) chemotherapy-associated toxicities were examined. The most common short-term toxicities (9.2–60%) included acute anemia, nausea, and neutropenia and the most common long-term toxicities (15–37%) included acute anemia, respiratory failure, pulmonary fibrosis, dehydration, neutropenia, nausea, and fever.⁷⁰ The discovery of activating mutations in the epidermal growth factor (EGF) receptor (EGFR) has led to the development of targeted lung cancer therapy. The main classes and targeted agents for NSCLC that are now being used include EGFR tyrosine kinase inhibitors (TKIs) like erlotinib (Tarceva[®]) and gefitinib (Iressa[®]), monoclonal antibodies against EGFR (cetuximab, Erbitux[®]). EGFR inhibitors have become the standard treatment in second- and third-line NSCLC therapy.^{71,72} Other molecules that have been targeted for the therapy of NSCLC include the vascular endothelial growth factor (VEGF), for which an inhibitor (bevacizumab, Avastin[®]) is approved by FDA, and the fusion protein EML4-ALK. Crizotinib is useful in a subset of NSCLC that is characterized by EML4-ALK expression, which was originally identified among relatively young patients with lung adenocarcinoma that never (or very lightly) smoked.⁷¹ Another recently established therapeutic strategy against NSCLC is based on gefitinib, which was analyzed as first-line therapeutic intervention in a study including 31 patients aged 75 y or older with advanced NSCLC harbouring EGFR mutations. The median progression-free survival was 12.3 mo. The most common adverse events were rash, diarrhea, and

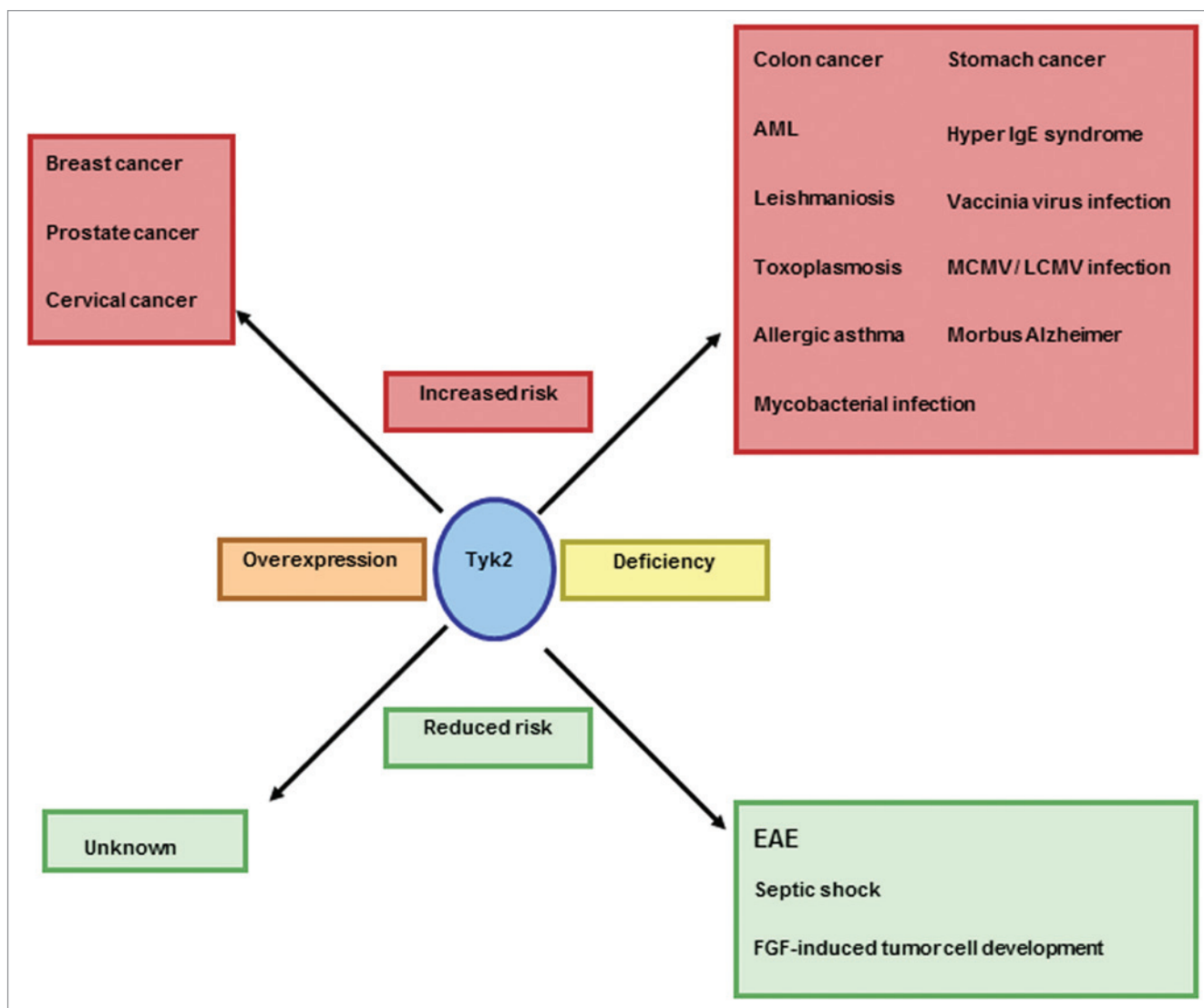


Figure 3. Involvement of TYK2 in disease. Altered expression of TYK2 is associated with several diseases. The Overexpression of TYK2 is associated with an increased risk of breast cancer, cervical cancer and prostate cancer, while there are no known diseases for which TYK2 overexpression reduces the risk. TYK2 deficiency leads to an increased susceptibility to a variety of disorders including colon cancer, acute myeloid leukemia (AML), allergic asthma, hyper IgE syndrome, leishmaniosis, toxoplasmosis, mycobacterial and viral infections, as well as Alzheimer disease. However, TYK2 deficiency is also associated with a reduced occurrence of experimental autoimmune encephalomyelitis (EAE), septic shock and oncogenesis. LCMV, lymphocytic choriomeningitis virus; MCMV, murine cytomegalovirus.

liver dysfunction.⁷³ However, the TALENT (1,172 chemo-naïve patients from 164 sites worldwide), TRIBUTE (1,059 patients) and INTACT-1/2 (2,130 patients) studies demonstrated that anti-EGFR (erlotinib or gefitinib) therapy combined with first-line standard chemotherapy (paclitaxel-carboplatin or gemcitabine-cisplatin) only ameliorates the survival of non-smokers.⁷⁴ The role of TYK2 in lung cancer is largely unknown. One possible involvement has been published by Gao et al., showing that mutations in the EGFR kinase domain lead to STAT3 activation in human lung adenocarcinomas. This effect was mainly mediated by IL-6 and hence also by TYK2 and JAK2.⁷⁵ Thus, TYK2 could represent an attractive target for the development of novel therapies for lung cancer.

Concluding Remarks

TYK2 is a molecule with diverse functions in the regulation of the immune system. Its inhibition diversely affects the occurrence of different diseases such as EAE, allergic asthma, rheumatoid arthritis, systemic lupus erythematosus and cancer (Fig. 3). Thus, the development of therapeutics targeting TYK2 should be precisely correlated with the disease under consideration and possible interactions with other diseases should be carefully evaluated. So far, one specific TYK2 inhibitor has been tested in vitro, while TYK2 overexpressing mutants have been found among human tumor samples. Much developmental work needs to be done if a specific TYK2 inhibitor is planned for clinical use. However, pan-JAK

inhibitors are already under clinical investigation for use in cancer therapy. Since these inhibitors near-to-completely block cell growth, tumor formation is inhibited. On one hand, murine data show that the blockade of TYK2 can decrease CD8⁺ T cell-mediated tumor surveillance and therefore promote cancer growth. On

the other hand, TYK2 inhibition might reduce the invasiveness of tumor cells upon the inhibition of FGF and EGF signaling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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