(Review Article)

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The JAK/STAT Signaling Pathway

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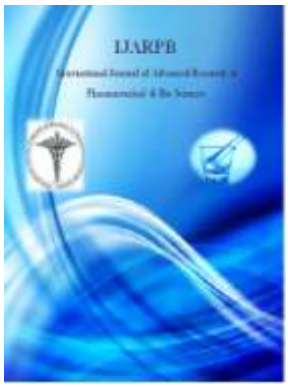

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ABSTRACT

The Janus kinase/signal transducers and activators of transcription (JAK/STAT)pathway is one of a handful of pleiotropic cascades used to transduce a multitude of signals for development and homeostasis in animals, from humans to flies.JAK activation stimulates cell proliferation,differentiation, cell migration and apoptosis.Thus it is involved in various processes such as hematopoiesis,adipogenesis,immune development.Mutation in this pathway constitutively activate or fail to regulate JAK signaling properly cause inflammatory disease, erythrocytosis, gigantism and an array of leukemias,and various other diseases.

KEYWORDS: The Janus kinase(JAK),signal transducers and activators of transcription(STAT),Tyrosine-protein kinase,Hematopoietic malignancies,Severe congenital neutropenia/acute myeloid leukemia,Benign erythrocytosis,Fanconi anemia

(Review Article)

INTRODUCTION

The Janus kinase/signal transducers and activators of transcription (JAK/STAT)pathway is one of a handful of pleiotropic cascades used to transduce a multitude of signals for development and homeostasis in animals, from humans to flies. In mammals, the JAK/STAT pathway is the principal signaling mechanism for a wide array of cytokines and growth factors. JAK activation stimulates cell proliferation,differentiation, cell migration and apoptosis. These cellular events are critical to hematopoiesis, immune development, mammary gland development and lactation, adipogenesis, sexually dimorphic growth and other processes. Predictably, mutations that reduce JAK/STAT pathway activity affect these processes[1].Conversely, mutations that constitutively activate or fail to regulate JAK signaling properly cause inflammatory disease, erythrocytosis, gigantism and an array of leukemias. Here we present a general overview of the JAK/STAT pathway and illustrate the primary mechanisms of activation and regulation of this essential signaling cascade.Mechanistically, JAK/STAT signaling is relatively simple, with only a few principal components[2].As described above, a variety of ligands and their receptors stimulate the JAK/STAT pathway. Intracellular activation occurs when ligand binding induces the multimerization of receptor subunits.For some ligands, such as erythropoietin and growth hormone, the receptor subunits are bound as homodimers while, for others, such as interferons and interleukins, the receptor subunits are heteromultimers.For signal propagation through either homodimers or heteromultimers, the cytoplasmic domains of two receptor subunits must be associated with JAK tyrosine kinases.JAKs are distinctive in that they havetandem kinase-homologous domains at the C-terminus.The first is a noncatalytic regulatory domain, whereas the second has tyrosine kinase activity.In mammals,the JAK familycomprisesfourmembers:JAK1,JAK2, JAK 3 and Tyk2. JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowingtrans-phosphorylation. The activated JAKs subsequently phosphorylate additional targets, including both the receptors and the major substrates,STATs. STATs are latent transcription factors that reside in the cytoplasm until activated. The seven mammalian STATs bear a conserved tyrosine residue near the C-terminus that is phosphorylated by JAKs. This phosphotyrosine permits the dimerization of STATs throughinteraction with a conserved SH2domain. Phosphorylated STATs enterthe nucleus by a mechanism that is dependent on importin (also called nucleoprotein interactor 1) and the Ran nuclear import pathway. Once in the nucleus, dimerized STATs bind specific regulatory sequences to activate or repress transcription of target genes. Thus the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response.

Janus kinase family

Janus kinase (**JAK**, or "Just another kinase") is a family of intracellular, non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway. They were initially named "just another kinase" 1 & 2 (since they were just two of a large number of discoveries in a PCR-based screen of kinases), but were

ultimately published as "Janus kinase". The name is taken from the two-faced Roman god of doorways, Janus, because the JAKs possess two near-identical phosphate-transferring domains.One domain exhibits the kinase activity while the other negatively regulates the kinase activity of the first.[3]

The Janus kinase family

There are four JAK family members:

- Janus kinase 1 (JAK1)
- Janus kinase 2 (JAK2)
- Janus kinase 3 (JAK3)
- Tyrosine kinase 2 (TYK2)

Transgenic mice that do not express JAK1 have defective responses to some cytokines such as interferon-gamma.[4] JAK1 and JAK2 are involved in type II interferon (interferon-gamma) signalling, whereas JAK1 and TYK2 are involved in type I interferon signalling. Mice that do not express TYK2 have defective natural killer cell function.[5]

Clinical significance

JAK inhibitors are under development for the treatment of psoriasis, rheumatoid arthritis, polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis.

The structure of JAKs

Domain structure of Janus kinases. JH = JAK homology domain.

JAKs range from 120-140 kDa in size and have seven defined regions of homology called Janus homology domain 1–7 (JH1-7). JH1 is the kinase domain important for the enzymatic activity of the JAK and contains typical features of a tyrosine kinase such as conserved tyrosines necessary for JAK activation (e.g. Y1038/Y1039 in JAK1, Y1007/Y1008 in JAK2, Y980/Y981 in JAK3, and Y1054/Y1055 in Tyk2). Phosphorylation of these dual tyrosines leads to the conformational changes in the JAK protein to facilitate binding ofsubstrate. JH2 is a *pseudokinase domain*, a domain structurally similar to a tyrosine kinase and is essential for a normal kinase activity yet lacks enzymatic activity. This domain may be involved in regulating the activity of JH1. The JH3-JH4 domain of JAKs shares homology with Src-homology-2 (SH2) domains. The amino terminal (NH_2) end (JH4-JH7) of Jaks is called a FERM domain (short for band 4.1 ezrin, radixin and moesin); this domain is also found in the focal adhesion kinase (FAK) family and is involved in association of JAKs with cytokine receptors and/or other kinases.[6]

Janus kinase 1 (JAK1)
Janus kinase(JAK1)

JAK1 is a human tyrosine kinase protein essential for signaling for certain type I and type II cytokines. It interacts with the common gamma chain (γc) of type I cytokine receptors, to elicit signals from the IL-2 receptor family (e.g. IL-2R, IL-7R, IL-9R and IL-15R), the IL-4 receptor family (e.g. IL-4R and IL-13R), the gp130 receptor family (e.g. IL-6R, IL-11R, LIF-R, OSM-R, cardiotrophin-1 receptor (CT-1R), ciliary neurotrophic factor receptor (CNTF-R), neurotrophin-1 receptor (NNT-1R) and Leptin-R). It is also important for transducing a signal by type I (IFN-α/β) and type II (IFN-γ) interferons, and members of the IL-10 family via type II cytokine receptors.[7]Jak1 plays a critical role in initiating responses to multiple major cytokine receptor families. Loss of Jak1 is lethal in neonatal mice, possibly due to difficulties suckling.Expression of JAK1 in cancer cells enables individual cells to contract, potentially allowing them to escape their tumor and metastasize to other parts of the body.[8]

Janus kinase 2 (JAK2)

Janus kinase 2 (commonly called **JAK2**) is a human protein that has been implicated in signaling by members of the type II cytokine receptor family (e.g. interferon receptors), the GM-CSF receptor family (IL-3R, IL-5R and GM-CSF-R), the gp130 receptor family (e.g. IL-6R), and the single chain receptors (e.g. Epo-R, Tpo-R, GH-R, PRL-R). JAK2 signaling is activated downstream from the prolactin receptor.[9] JAK2 gene fusions with the TEL(ETV6) (TEL-JAK2) and PCM1 genes have been found in leukemia patients. Further, mutations in JAK2 have been implicated in polycythemia vera, essential thrombocythemia, and other myeloproliferative disorders.[10] This mutation, a change of valine to phenylalanine at the 617 position, appears to render hematopoietic cells more sensitive to growth factors such as erythropoietin and thrombopoietin. Loss of Jak2 is lethal by embryonic day 12 in mice.[11]

Janus kinase 3 (JAK3)

Tyrosine-protein kinase JAK3 is an enzyme that in humans is encoded by the *JAK3*gene.[12][13] JAK3 encodes Janus kinase 3, a tyrosine kinase that belongs to the Janus family. JAK3 functions in signal transduction and interacts with members of the STAT (signal transduction and activators of transcription) family. JAK3 is predominantly expressed in immune cells and transduces a signal in response to its activation via tyrosine phosphorylation by interleukin receptors. Mutations that abrogate Janus kinase 3 function cause an autosomal SCID (severe combined immunodeficiency disease).[14]

Since **JAK3** expression is restricted mostly to hematopoietic cells, its role in cytokine signaling is thought to be more restricted than other JAKs. It is most commonly expressed in T cells and NK cells, but has been induced in otherleucocytes, including monocytes. Jak3 is involved in signal transduction by receptors that employ the common gamma chain (γC) of the type I cytokine receptor family (e.g. IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R).[4] Mutations of JAK3 result in severe combined immunodeficiency (SCID). Mice that do not express JAK3 have T-cells and B-cells that fail to respond to many cytokines.[15]

Tyrosine kinase 2 (TYK2)

Non-receptor tyrosine-protein kinase TYK2 is an enzyme that in humans is encoded by the *TYK2*gene.Tyk2 was the first member of the JAK family that was described (the other members are JAK1, JAK2, and JAK3).It has been implicated in IFN-α, IL-6, IL-10 and IL-12 signaling.

Function.[16]

(JAKs) protein families. This protein associates with the cytoplasmic domain of type I and type II .This gene encodes a member of the tyrosine kinase and, to be more specific, the Janus kinases II cytokine receptors and promulgate cytokine signals by phosphorylating receptor subunits. It is also component of both the type I and type III interferon signaling pathways. As such, it may play a role in anti-viral immunity.

Cytokines play pivotal roles in immunity and inflammation by regulating the survival, proliferation, differentiation, and function of immune cells, as well as cells from other organ systems.[4] Hence, targeting cytokines and their receptors is an effective means of treating such disorders. Type I and II cytokine receptors associate with Janus family kinases (JAKs) to effect intracellular signaling. Cytokines including interleukins, interferons and hemopoietins activate the Janus kinases, which associate with their cognate receptors.

The mammalian JAK family has four members: JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). The connection between Jaks and cytokine signaling was first revealed when a screen for genes involved in interferon type I (IFN-1) signaling identified Tyk2 as an essential element, which is activated by an array of cytokine receptors.Tyk2 has broader and profound functions in humans than previously appreciated on the basis of analysis of murine models, which indicate that Tyk2 functions primarily in IL-12 and type I-IFN signaling. Tyk2 deficiency has more dramatic effects in human cells than in mouse cells. However, in addition to IFN-α and -β and IL-12 signaling, Tyk2 has major effects on the transduction of [IL-23, IL-1AT 0, and IL-6 signals. Since, IL-6 signals through the gp-130 receptor-chain that is common to a large family of cytokines, including IL-6, IL-11, IL-27, IL-31, oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin 1, cardiotrophin-like cytokine, and LIF, Tyk2 might also affect signaling through these cytokines. Recently, it has been recognized that IL-12 and IL-23 share ligand and receptor subunits that activate Tyk2. IL-10 is a critical anti-inflammatory cytokine, and IL-10$^{-/-}$ mice suffers from fatal, systemic autoimmune disease.

Tyk2 is activated by IL-10, and its deficiency affects the ability to generate and respond to IL-10. Under physiological conditions, immune cells are, in general, regulated by the action of many cytokines and it has become clear that cross-talk between different cytokine-signalling pathways is involved in the regulation of the JAK–STAT pathway.[17]

General functions of the JAK family

Since members of the type I and type II cytokine receptor families possess no catalytic kinase activity, they rely on the JAK family of tyrosine kinases to phosphorylate and activate

downstream proteins involved in their signal transduction pathways. The receptors exist as paired polypeptides thus exhibiting two intracellular signal-transducing domains. JAKs associate with a proline-rich region in each intracellular domain, which is adjacent to the cell membrane and called a box1/box2 region. After the receptor associates with its respective cytokine/ligand it goes through a conformational change, bringing the two JAKs close enough to phosphorylate each other. The JAK autophosphorylation induces a conformational change within itself enabling it to transduce the intracellular signal by further phosphorylating and activating transcription factors called STATs. The activated STATs dissociate from the receptor and form dimers before translocating to the cell nucleus where they regulate transcription of selected genes.Some examples of the molecules that utilize JAK/STAT signaling pathway are colony-stimulating factor, prolactin, growth hormone, and many cytokines.[18]

Many receptors signal through a small number of JAKs

Cytokine receptors signal through two types of pathways: theJAK-STAT pathway and other pathways that usually involvethe activation of the MAP kinase cascade. Although the latterwill not be discussed here, it is worth noting that elegant geneticstudies have demonstrated the importance of these pathways invarious pathological systems (19–20). There are now _36 cytokinereceptor combinations that respond to _38 cytokines(counting the type I IFNs as one because they all signal throughthe IFN-__R). Different cells and tissues express distinct receptor combinations that respond to cytokine combinationsunique to the microenvironment or systemic response of theorganism. Hence, at any given time, a single cell may integratesignals from multiple cytokine receptors. Genetic studies haveestablished that the cytokine receptor system is restrictive inthat different classes of receptors preferentially use one JAK orJAK combination (7): receptors required for hemopoietic celldevelopment and proliferation use JAK2, common _-chain receptorsuse JAK1 and JAK3 whereas other receptors use onlyJAK1 (Fig. 1). Unexplained is the selective use of these combinations:why the IFN-_R rigidly uses the JAK1, JAK2 combinationis unknown as is the restricted use of TYK2. Comparedwith JAK1–3, TYK2 is unusual in that loss of function mutationsin the mouse have shown obligate, but not absolute, requirementsin IFN-__R and IL-12R signaling . In contrast,human TYK2 seems to be essential for signaling througha broader range of cytokine receptors .The preferential association of JAKs to certain receptorclasses raises several issues. First, how did the JAK-receptorcombinations evolve? Because the number of receptors is relativelylarge, why has the number of JAKs remained small? Whyn have the combinations of JAK pairs also remained small giventhat there are 10 possible combinations that can be used (Fig.1)? Second, how flexible is the cytokine receptor-JAK pair?That is, can receptors be engineered for interchangeable JAKuse, or is a given JAK combination fixed for a specific receptorclass? For example, can JAK1, JAK3, or TYK2 activate erythropoietinreceptor (EpoR) signaling (if so engineered) or is mJAK2 obligatory for signaling? These questions allude to a fundamentalissue that concerns the function of the JAK in cytokinereceptor activation: if the only function of

the JAKs is tophosphorylate tyrosine resides on the cytoplasmic domain ofthe receptors, then it should be possible to trade JAK-receptorpairs. If these receptors retain identical downstream gene expressionprofiles, then the signal generated by the JAK is genericand functions primarily to activate the receptor . Conversely,it is also possible that each receptor-JAK combination retainscrucial specificity functions and swapping, for example, JAK1for JAK2 on the EpoR will modify or destroy a specific functionin erythrogenesis. These questions can be addressed experimentallyby replacing one preferred JAK binding site for another ingenes encoding different receptors. The EpoR is a good test examplebecause the activity of the receptor and its signaling pathwayis essential for life and erythropoiesis is readily assayed.[20]

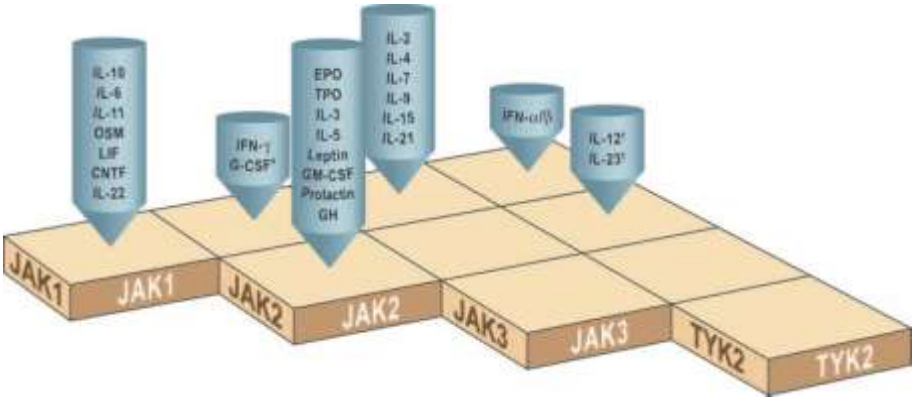

FIGURE 1: The majority of cytokine receptors use three JAK combinations. Shown are well-studied cases where JAK usage by each cytokine receptor has been established by genetic and biochemical studies. Exceptions shown are the G-CSFR (_) where it is currently unclear whether both JAK1 and JAK2 are required together. Additionally, the IL-12R (†) and IL-23R (†) require TYK2 but the requirement for JAK2 has not been definitively determined. Receptors that use JAK2 and JAK3, JAK3 alone, TYK2 alone, or JAK3 and TYK2 have not been described.

STAT FAMILY

The STAT protein (Signal Transducer and Activator of Transcription, or Signal Transduction And transcription) regulates many aspects of growth, survival and differentiation in cells. The transcription factors of this family are activated by Janus kinase (JAK) and dysregulation of this pathway is frequently observed in primary tumours and leads to increased angiogenesis, enhanced survival of tumours and immunosuppression. Gene knockout studies have provided evidence that STAT proteins are involved in the development and function of the immune system and play a role in maintaining immune tolerance and tumour surveillance.

The first two STAT proteins were identified in the interferon system. There are seven mammalian STAT family members which have been identified: STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5A and STAT5B), and STAT6. STAT1 homodimers are involved in type II interferon signalling, and bind to the GAS (Interferon-Gamma Activated Sequence) promoter to induce expression of ISG (Interferon

Stimulated Genes). In type I interferon signaling, STAT1-STAT2 heterodimer combines with IRF9 (Interferon Response Factor) to form ISGF3 (Interferon Stimulated Gene Factor), which binds to the ISRE (Interferon Stimulated Response Element) promoter to induce ISG expression Function. STAT proteins were originally described as latent cytoplasmic transcription factors that require phosphorylation for nuclear retention. The unphosphorylated STAT proteins shuttles between cytosol and the nucleus waiting for its activation signal. Once the activated transcription factors reaches the nucleus it binds to consensus DNA-recognition motif called gamma activated sites (GAS) in the promoter region of cytokineinducible genes and activates transcription of these genes.

Activation

Extracellular binding of cytokines induces activation of the intracellular Janus kinase that phosphorylates a specific tyrosine residue in the STAT protein which promotes the dimerization of STAT monomers via their SH2 domain. The phosphorylated dimer is then actively transported in the nucleus via importin a/b and RanGDP complex. Once inside the nucleus the active STAT dimer binds to cytokine inducible promoter regions of genes containing gamma activated site (GAS) motif and activate transcription of these genes. The STAT protein can be dephosphorylated by nuclear phosphatases which leads to inactivation of STAT and the transcription factor becomes transported out of the nucleus by exportin crm1/RanGTP.[21]

STAT 1

STAT1 is a member of the Signal Transducers and Activators of Transcription family of transcription factors. STAT1 is involved in upregulating genes due to a signal by either type I, type II or type IIIinterferons. In response to IFN-γ stimulation, STAT1 forms homodimers or heterodimers with STAT3 that bind to the GAS (Interferon-Gamma Activated Sequence) promoter element; in response to either IFN-α or IFN-β stimulation, STAT1 forms a heterodimer with STAT2 that can bind the ISRE (Interferon Stimulated Response Element) promoter element.[1] In either case, binding of the promoter element leads to an increased expression of ISG (Interferon Stimulated Genes). Expression of STAT1 can be induced with diallyl disulfide, a compound in garlic.[22]

STAT 2

Signal transducer and activator of transcription 2 is a protein that in humans is encoded by the STAT2gene.[1][2]The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. In response to interferon (IFN), this protein forms a complex with STAT1 and IFN regulatory factor family protein p48 (ISGF3G), in which this protein acts as a transactivator, but lacks the ability to bind DNA directly. Transcription adaptor P300/CBP (EP300/CREBBP) has been shown to interact specifically with this protein, which is thought to be involved in the process of blocking IFN-alpha response by adenovirus.[22]

STAT 3

Signal transducer and activator of transcription 3 also known as STAT3 is a transcription factor which in humans is encoded by the STAT3gene.

Function

The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by receptor-associated kinases and then form homo- or heterodimers that translocate to the cell nucleus, where they act as transcription activators. This protein is activated through phosphorylation of two residues, tyrosine 705 and serine 727, in response to various cytokines and growth factors including interferons, epidermal growth factor, Interleukin 5, Interleukin-6, hepatocyte growth factor, leukemia inhibitory factor (LIF), bone morphogenetic protein 2 and also the hormone leptin. STAT3 mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein. Three alternatively spliced transcript variants encoding distinct isoforms have been described.

The binding of Interleukin 6—family cytokines (including IL-6, oncostatin M and leukemia inhibitory factor) to the gp130 receptor triggers STAT3 phosphorylation by JAK2. Epidermal growth factor receptor and certain other receptor tyrosine kinases, such as c-MET, phosphorylate STAT3 in response to their ligands. STAT3 is also a target of the c-src non-receptor tyrosine kinase.

STAT3-deficient mouse embryos cannot develop beyond embryonic day 7, when gastrulation begins.It appears that at these early stages of development, STAT3 activation is required for self-renewal of embryonic stem cells (ESCs). Indeed, LIF, which is supplied to murine ESC cultures to maintain their undifferentiated state, can be omitted if STAT3 is activated through some other means.

STAT3 is essential for the differentiation of the TH17 helper T cells, which have been implicated in a variety of autoimmune diseases.[24]

Clinical significance

Loss-of-function mutations in the STAT3 gene result in Hyperimmunoglobulin E syndrome, associated with recurrent infections as well as disordered bone and tooth development.

Constitutive STAT3 activation is associated with various human cancers and commonly suggests poor prognosis. It has anti-apoptotic as well as proliferative effects.

Dual role in cancer

STAT3 can promote oncogenesis by being constitutively active through various pathways as mentioned elsewhere. Very recently a tumor suppressor role of STAT3 has also been reported[12]. In this report on human glioblastoma tumor, or brain cancer, STAT3 was shown to have an oncogenic or a tumor suppressor role depending upon the mutational background of the tumor. A direct connection between the PTEN-Akt-FOXO axis (suppressive) and the leukemia inhibitory factor receptor beta (LIFRbeta)-STAT3 signaling pathway (oncogenic) was shown

STAT 4

Stat4 was first isolated by two groups using degenerative-PCR or low-stringency hybridization, both of which were based on homology with the SH2 domain of other STAT proteins. To date, most studies of Stat4 have been focused on the functional analysis of this molecule in T cells, because Stat4 is mainly phosphorylated by IL-12-mediated signaling pathway in T cells. Here we will first describe the signal transduction pathway of IL-12 briefly, and then discuss the role of Stat4 in the functional differentiation and proliferation of T cells.Stat4 expression is restricted in myeloid cells, thymus and testis. In human T cells, Stat4 expression is dramatically induced by activation with PHA (phytohemaglutinin), although its expression is very low in resting T cells . IL-12 is the major cytokine that can activate Stat4, resulting in its tyrosine phosphorylation, in both human and mouse . The IL-12 receptor is composed of two chains, termed IL-12R 1 and IL-12R 2, and ligand binding results in heterodimer formation and activation of the receptor associated JAK kinases, Jak2 and Tyk2 . Stat4 is phosphorylated by these tyrosine kinases, homodimerizes via its SH2 domain, and translocates into nucleus where it can recognize traditional N3 STAT target sequences in IL-12 responsive genes.

Although IL-12 appears to be the predominant activator of Stat4, Stat4 can also be phosphorylated in response to IFN- stimulation through activation of Jak1 and Tyk2, but this has only been observed in human cells (Cho et al., 1996). Additionally, recent studies in human vascular smooth muscle and endothelial cells demonstrated that the urokinase-type plasminogen activator (uPA) receptor is associated with Jak1 and Tyk2, and uPA stimulation of these cells resulted in Stat4 phosphorylation . IL-17 can also activate Stat4 in human monocytic leukemia cell lines. Furthermore, IL-2 can induce Jak2 and Stat4 activation in NK cells but not in T cells.

IL-12 is capable of inducing a mitogenic response in some cell types. A role for Stat4 in transducing this signal was demonstrated in Stat4-deficient mice . Stat4-deficient lymphocytes were specifically defective in their ability to proliferate in response to IL-12. Additionally, Stat-4-deficient lymphocytes, like Stat6-deficient lymphocytes, were unable to downregulate p27kip protein levels after cytokine stimulation . These data suggest that STAT proteins, as a general mechanism, may control cytokine-mediated cell proliferation by regulating the expression of cell cycle inhibitors which are critically involved in cell cycle progression[24].

STAT 5a

Signal transducer and activator of transcription 5A is a protein that in humans is encoded by the STAT5Agene.STAT5Aorthologs have been identified in several placentals for which complete genome data are available.

The protein encoded by this gene is a member of the STAT family of transcription factors. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein is activated by, and mediates the responses of many cell ligands, such as IL2, IL3, IL7 GM-CSF, erythropoietin, thrombopoietin, and different growth hormones. Activation of this protein in myeloma and

lymphoma associated with a TEL/JAK2 gene fusion is independent of cell stimulus and has been shown to be essential for the tumorigenesis. The mouse counterpart of this gene is found to induce the expression of BCL2L1/BCL-X(L), which suggests the antiapoptotic function of this gene in cells[25].

STAT 5b

Signal transducer and activator of transcription 5B is a protein that in humans is encoded by the STAT5Bgene.STAT5B orthologs have been identified in most placentals for which complete genome data are available.

The protein encoded by this gene is a member of the STAT family of transcription factors. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein mediates the signal transduction triggered by various cell ligands, such as IL2, IL4, CSF1, and different growth hormones. It has been shown to be involved in diverse biological processes, such as TCR signaling, apoptosis, adult mammary gland development, and sexual dimorphism of liver gene expression. This gene was found to fuse to retinoic acid receptor-alpha (RARA) gene in a small subset of acute promyelocytic leukemias (APML). The dysregulation of the signaling pathways mediated by this protein may be the cause of the APML[26].

STAT 6

STAT6 is a human gene. The protein encoded by this gene is a member of the STAT family of transcription factors.[1][2] In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein plays a central role in exerting IL4 mediated biological responses. It is found to induce the expression of BCL2L1/BCL-X(L), which is responsible for the anti-apoptotic activity of IL4. Knockout studies in mice suggested the roles of this gene in differentiation of T helper 2 (Th2), expression of cell surface markers, and class switch of immunoglobulins[27].

Core versus cell-type specific STAT signaling

Microarray experiments designed to monitor changes in geneexpression induced by JAK-STAT signaling have revealed thatboth cell-type specific transcription and core, or stereotypic,mRNA profiles are induced by activated cytokine receptors indifferent cell types (Fig. 2). For example, IFN-_, via STAT1,induces the expression of a similar cohort of genes regardless ofthe cell type tested. These genes are often termed the “IFNsignature” and overlap with the gene expression pattern inducedby IFN-__ signaling that also involves STAT1, in cooperationwith STAT2 and IRF9. The IFN signature is readilyobserved in microarray experiments and is indicative of STAT1activity. The STAT6 pathway activated by IL-4 or IL-13 providesan example of a cell-type specific response. IL-4-regulatedgenes in T cells have a distinct signature compared with IL-4/IL-13 signaling in macrophages or other non-lymphocytes(28 –29). In the latter, genes such as *Arg1* (encoding arginase 1)are often induced

_100-fold but are silent in T cells .Collectively these data argue that STATs activate defined genesets, depending on their genomic accessibility, and possibly oncofactors that further refine gene expression profiles. STAT3signaling illustrates a more complex system and will be discussedbelow to illustrate the distinctions between IL-6 and IL-10 signaling[30].

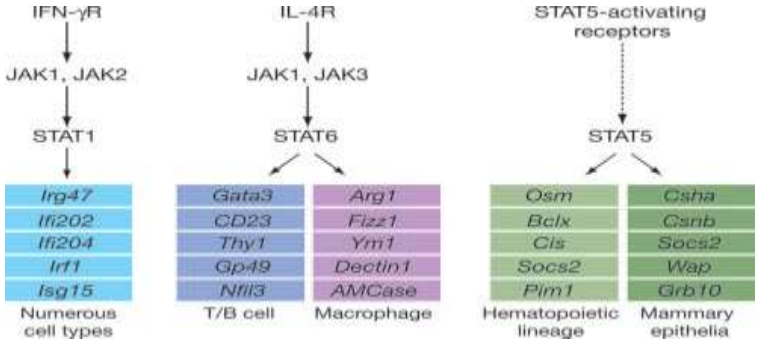

FIGURE 2: Core signaling by STATs. Representative examples of gene expression induced by STAT signaling in different tissues. The examples were extracted and edited from numerous microarray and empirical studies.

JAK-STAT activation mechanism

The current model of JAK-STAT signaling holds that cytokine receptorengagement activates the associated JAK combination,which in turn phosphorylates the receptor cytoplasmic domainto allow recruitment of a STAT, which in turn is phosphorylated,dimerizes and moves to the nucleus to bind specific sequencesin the genome and activate gene expression. Cytoplasmicdomains of cytokine receptors associate with JAKs via JAKbinding sites located close to the membrane[31]. The postulatedrole of JAKs in trafficking or chaperoning the receptors to thecell surface is debated. Regardless of the when and wherecytokine receptors and JAKs associate, their close apposition at the membrane is required to stimulate the kinase activity of theJAK following cytokine binding[32]. At this stage in the activationof the pathway, we understand next to nothing about the structuralbasis of the JAK-receptor interaction, how receptor intracellulardomains reorient upon cytokine binding and physicallycontact the JAK to receive the phosphorylation modification.JAK-mediated phosphorylation of the receptor creates bindingsites for the Src homology 2 (SH2) domains of the STATs.STAT recruitment is followed by tyrosine, and in some cases,serine phosphorylation on key residues (by the JAKs and otherclosely associated kinases) that leads to transit into the nucleus.This brief summary of the activation of the JAK-STAT pathwayomits numerous unresolved details: the STAT monomer todimer transition has been questioned, as has the role of phosphorylationin dimerization and nuclear transit[33]. Furthermore,it is unclear how many configurations of STAT homoandheterocomplexes are present in cells before, during, and aftercytokine stimulation. We

do not understand the detailedstructural basis for the preference of one SH2 domain fora given receptor, and we have little knowledge of how othernon-JAK kinases are recruited to the receptors and phosphorylatethe STATs.

Negative regulation of the JAK-STAT signal

Many biochemical processes conspire to regulate the JAKSTATpathway, including phosphorylation-mediated autoinactivationof the FERM domain of the JAKs, loss of receptornumbers at the surface, dissociation of JAKs from a receptor,heterodimer competition and transport of STATs out of thenucleus. I will discuss two mechanisms of JAK-STAT regulationwhose biochemical processes are mechanistically unclearbut likely play central roles in negative regulation of the STATactivation process, and therefore translate into large effects atthe level of gene regulation.SOCS proteins are induced by cytokines and other stimuliand function as negative feedback inhibitors of cytokine receptorsignaling. The prevailing hypothesis for SOCS function hasfocused on an initial receptor binding step, mediated by theSH2 domain of the SOCS protein, followed by a second step involving the activity of the SOCS box, which forms a complexwith proteins involved in ubiquitin E3 ligase activity .SOCS1, SOCS2, and SOCS3 have been found to have surprisinglyselective essential functions in regulating cytokine signaling. The genetic studies raise a complex issue in that lossof-function studies can only identify the non-redundantfunctions of each SOCS protein. Other targets of the SOCSproteins that are not cytokine receptors have been proposed.The functions of these interactions are not readily determinedby genetics and their roles normal cellular physiology have yetto be firmly established.The functions of the SH2 domain and the SOCS box can beseparated and, at least for SOCS1, the key inhibitor of theIFN-γ R. Mice lacking SOCS1 die rapidly from excessive IFN -γ signaling: a phenotype that can be rescuedin multiple ways that reduce the amounts of IFN-γ orIFN-γsignaling . However, mice lacking the SOCS box ofSOCS1 but retaining the SH2 domain that binds to theIFN-γR are partially protected from the toxic effects of unregulatedIFN-γ signaling . This suggests that both SH2 andSOCS box domains have inhibitory activity toward cytokinesignaling. How the two inhibitory signals delivered by a SOCSprotein are integrated with other negative signals to block cytokinereceptor signaling are unknown and require further study.Although impressive inroads into understanding the functionsand specificities of the SOCS proteins have been made,one major question is outstanding: what are the substrates of theubiquitin E3 ligase activity of each SOCS protein? A simplemodel for SOCS function is that binding of the SOCS SH2domain anchors the protein complex close to the receptor, increasingthe effective concentration of the complex to where it isneeded. But what then are the targets? Although we suspectedthat the receptor chains themselves could be the target for ubiquitinationand degradation, we could not find compelling evidenceto support this idea in the case of the SOCS3-gp130 interaction. Similarly, it is difficult to imagine how SOCSproteins could selectively and specifically block JAK signaling atone receptor class while leaving other receptors using the sameJAK unaffected, although many investigators favor this hypothesisas a partial solution to the problem of identifying SOCSsubstrates .Since the overall

biochemical activity of SOCS proteins is toreduce the output of activated cytokine signaling, maybe theSOCS proteins direct the ubiquitination of the phosphorylatedSTATs, and thereby their degradation, reducing the amplitudeof the STAT signal. Such a mechanism would occur in a dynamicway, because STATs are continuously recruited to activatedreceptors during signaling, while selected SOCS proteinsare continuously made in response to STAT activation. Earlywork, preceding the discovery of the SOCS proteins, noted thatproteasome inhibition increased cytokine receptor signaling,implicating ubiquitin-mediated degradation in blocking theJAK-STAT pathway . Validation of this idea requiressensitive tests to measure percentages of STAT proteins that aremodified by ubiquitination. However, the identity of the relevantSOCS substrates is an intrinsically complex biochemicalproblem that will not be solved easily.Several investigators have observed that STAT DNA bindingactivity can rapidly (preceding the induction of the SOCS proteins)be inhibited by other receptor signaling systems. For example,STAT3 DNA binding activity is blocked by cosignalingthrough the IL-1R or TLR4, which does not use the JAK-STATsystem , suggesting rapid inhibitory effects on STATactivity seems to occur in a membrane proximal way. Thesedata argue that kinases, phosphatases, and other enzymes can berecruited in the vicinity of cytokine receptors and influencetheir function. These poorly understood pathways likely havesignificant effects during inflammatory responses where coincidentTLR, TNF-_R and IL-1R signaling are acting. For example,in the example of IL-10 versus IL-6 signaling that follows,LPS seems to affect early signaling from the IL-6R but not theIL-10R.[34-35].

INVOLVEMENT OF THE JAK-STAT PATHWAY IN DISEASE

Jaks, Stats, and hematopoietic diseases

The wide use of the Jak-Stat pathway by hematologically important factors, the severity of artificially disrupting the Jak-Stat pathway on hematopoiesis, and the number of key genes with Stat-response elements already provides some appreciation of the importance of this pathway in hematopoiesis and the regulation of hematopoietic cell function. We will now summarize the studies showing that several diverse hematopoietic disorders exhibit perturbations in the Jak-Stat pathway. Indeed, in a number of these cases, experiments have directly implicated the altered Jak or Stat signaling, or both, in the pathogenesis of the disease. Such molecular investigations provide a foundation on which to build an understanding of these conditions and a framework for rational improvements in therapy.

Hematopoietic malignancies

Aberrant activation of Jaks and Stats

The most direct evidence implicating dysregulation of the Jak-Statpathway in hematopoietic malignancies was the identification ofTel-Jak2 fusions in lymphoid and myeloid leukemias.In earlyB-precursor acute lymphoblastic leukemia, t(9;12)(p24;p13) translocationswere responsible, whereas in the case of atypical chronicmyeloid leukemia there was a complex t(9;15;12)(p24;q15;p13)translocation. In each case, the helix-loop-helix oligomerizationdomain of the transcription factor Tel is fused to the catalytic JH1domain of Jak2 (Figure 2), which

leads to constitutive associationand hence activation of the kinase and constitutive activation ofStat proteins.123,124 However, Jaks and Stats are also known to beconstitutively activated in hematopoietic cells transformed bydiverse oncogenic tyrosine kinases , as well as in a varietyof lymphomas and leukemias, including those transformed byoncogenic viruses. For the oncogenic tyrosine kinases,the activation of Stats may be direct or occur through Jaks, whereasthe oncogenic viruses activate a number of cytoplasmic kinases tomediate the constitutive Stat activation observed[37,38].

Evidence for Jak-Stat involvement

Although the data above are suggestive of a positive role forconstitutive activation of the Jak-Stat pathway in leukemia, theresults are largely correlative. However, other studies have providedmore direct evidence for this hypothesis.For example, overexpression of a Tel-Jak2 fusion is sufficient to render Ba/F3cells factor-independent,and mice transplanted with retrovirusexpressing this fusion develop a fatal mixed myeloproliferativeand T-cell lymphoproliferative disorder with a latency of 2 to 10weeks[39].In addition, 2 mutants of the *Drosophila* Jak kinase havebeen identified that lead to leukemia-like defects through hyperactivationof the kinase. Murine homologues of this Jak mutanthave been further shown to induce leukemia in mice. Finally,inhibition of constitutive Jak2 phosphorylation in primary pre-Bleukemic cells with the Jak2 inhibitor AG490 is able to inhibit cellproliferation. However, other studies suggest that the constitutiveJak activation seen in transformed cells is not actually requiredfor transformation. Thus, dominant-negative Jaks are unable toinhibit either Stat5 activation or factor-independent cell proliferationinduced by Bcr-Abl. In addition, v-Src can directly activateStat3,whereas the Herpesvirus Tip protein co-associates Lck andStat3, leading to constitutive Stat3 activation in T cells transformedby this virus, suggesting that in these cases Jaks are alsosuperfluous.In contrast, numerous recent studies have provided strongevidence for a role of Stats in the transformation process. Forexample, a dominant-negative Stat5 was able to inhibit apoptosisresistant,growth factor-independent proliferation and leukemicpotential of Bcr-Abl transformed cells and of the growthfactor-independent colony formation of primary mouse bonemarrow progenitor cells transduced with Bcr-Abl retrovirus.Inaddition, a constitutively active Stat5 mutant could restore thesefunctions to a mutant Bcr-Abl deficient in Stat activation.Similarly, the abrogation of IL-3 dependence of myeloid cells byv-Src requires the SH2 and SH3 domains, which specifies theactivation of Stats,and dominant-negative Stat3 has been shownspecifically to block v-Src transformation in other cell systems. Furthermore, studies in multiple myeloma cells thatshow constitutive Stat activation have revealed that dominantnegativeStat3 induces apoptosis,again implicating Stat3 in thetransformation process. Finally, a constitutively active mutant ofStat5 is sufficient to induce factor independence of Ba/F3 cells.Thus, constitutive Stat activation appears necessary, and perhapssufficient, for the transformation process.The results of the above studies imply that a permanentalteration in the genetic program of transformed cells, achieved bythe constitutive activation of Stat proteins, is a critical step in thetransformation process. Recent studies have

begun to shed light onthose changes that may be important. Thus, constitutive expression of Jak2 in Ba/F3 cells has been shown to lead to the induction ofBcl-2, resulting in delayed cell death,138 but the constitutivelyactivated Stat3 observed in bone marrow mononuclear cells frompatients with multiple myeloma also confers resistance to apoptosis,this time through the induction of Bcl-xL. Identification ofother genes involved in the transformation process remains animportant goal for future research[40].

Other hematologic disorders

Alterations in the Jak-Stat pathway have been associated either directly or indirectly with other hematologic disease states.

Severe combined immunodeficiency

In the most common form of severe combined immunodeficiency (SCID), X-linked SCID, both cellular and humoral immunity are severely affected: T-cell development is arrested in the thymic cortex, there is an almost complete lack of circulating T lymphocytes,and, though B lymphocytes are present, they do not undergo class switching.In X-linked SCID, mutations have been identified in the gene encoding the common g chain (gc), a constituent of a number of cytokine receptor complexes.Although these mutations occur at multiple positions, all mutant receptors are defective in the activation of Jak3.Indeed, *Jak3* knockout mice display a SCID phenotype that is virtually indistinguishable from that of gc null mice.Moreover, in a less common autosomal-recessive form of SCID, patients have been reported with inactivating mutations in the *Jak3* gene itself.Together these findings show that abrogation of the Jak-Stat pathway is sufficient to account for SCID in humans[41].

Severe congenital neutropenia/acute myeloid leukemia

Patients with severe congenital neutropenia (SCN) exhibit a severe reduction in circulating neutrophils and a maturation arrest of bone marrow progenitor cells at the promyelocyte/myeloid stage.Such patients have an increased risk for myelodysplasia, acute myeloid leukemia, or both, and a poor prognosis for survival.A subset of patients with SCN has been identified with acquired nonsense mutations in the gene encoding the G-CSF receptor, which truncate its carboxyl-terminus.This subset has a strong (around 50%) predisposition to acute myeloid leukemia. Mice carrying a similar G-CSF-R truncation also show reduced basal levels of circulating neutrophils, but on continuous G-CSF treatment, neutrophil counts become elevated to above those of wild-type controls because of the increased proliferation of myeloid progenitors.This suggests that the G-CSF-R truncation may contribute to SCN and to the subsequent development of acutemyeloid leukemia in these patients. Bone marrow cells from these mutant mice show reduced Stat3 activation in response to G-CSF, even under saturating conditions.

In addition, there is an altered dose-response of Stat3 compared to Stat5 activation, such that at lower G-CSF concentrations the Stat3 deficiency is even more pronounced, a result confirmed in myeloid 32D cells.Because Stat3 appears indispensable for differentiation responses to G-CSF, the reduced Stat3:Stat5 ratio in cells with truncated receptors at low G-CSF concentrations

may contribute to the reduced maturation observed. In addition, molecular mechanisms have been identified recently that explain the hyperproliferative function of truncated G-CSF-R. Such receptors show defective internalization compared with wild-type receptors,and have a concomitant extension in the activation of Stats, particularly Stat5,consistent with a previous report of enhanced Jak2 activation in patients with SCN .

It has recently been shown in 32D cells expressing truncated G-CSF-R that dominant-negative Stat5 inhibits whereas dominant-negative Stat3 actually enhances G-CSF–mediated growth, implicating perturbed Stat5 activation as a keym molecular determinant of the hyperproliferative responses elicited from truncated G-CSF-R (Ward et al, manuscript in preparation). In addition, a novel G-CSF-R mutation has been identified in a patient with SCN who was unresponsive to G-CSF therapy—in this case Stat5 activation was substantially reduced, again consistent with an important role of Stat5 in controlling proliferative responses to G-CSF[42].

Benign erythrocytosis

Benign erythrocytosis is a dominant autosomal condition characterized by a mild increase in red blood cell counts and normal serum levels of erythropoietin because of hypersensitivity to erythropoietin. In addition, there is an increased and a sustained activation of Jak2 and Stat5 after erythropoietin stimulation. A number of pedigrees have been identified, all of which lead to erythropoietin (EPO)-R truncations that invariably result in the loss of the binding site for SHP-1 at Tyr 449 of the EPO-R.Because SHP-1 is a negative regulator of Jak2 activation by EPO, it appears that lack of SHP-1 activation is responsible for the altered Jak-Stat kinetics and enhanced EPO responses in these patients[43].

Fanconi anemia

Fanconi anemia (FA) is an autosomal recessive chromosome instability syndrome characterized by progressive bone marrow failure and an increased susceptibility to malignancy. The FA group C gene (FAC) has been identified, with its disruption leading to profound hypersensitivity of hematopoietic precursor cells to IFN-g in mice and in patients with FA group C. This appears to be the result of sustained Stat1 activation leading to apoptosis of these cells. Other researchers have reported that the FAC protein is involved in the recruitment of Stat1 to the IFN-g receptorcomplex, which further suggests that perturbed Stat1 activation contributes to the phenotype of this disease[44].

OBESITY

In leptin-resistant tissue (e.g., hypothalamic cell illustrated), serum leptin interacting proteins (SLIPs) and soluble leptin receptor (SLR) may bind circulating adipose-secreted leptin and inhibit its action.

Free leptin engages the long form of its receptor (Ob-Rb), which homodimerizes. Intracellularly, activated janus kinase 2 (JAK2) phosphorylates a specific tyrosine docking site (Tyr1138) on Ob-Rb. Signal transduction and translation protein 3 (STAT3) recognizes and binds to activated Tyr1138 via its src homology 2 (SH2) domain.

The Ob-Rb/JAK2 complex activates STAT3, which homodimerizes, then translocates to the nucleus to modulate gene transcription. STAT3

up-regulates expression of suppressor-of cytokine-signaling-3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B), which block JAK2 phosphorylation. It is thought that central leptin resistance promotes obesity, driving greater hyperleptinemia. (B) In nonleptinresistant tissue (e.g., immune cell illustrated) exposed to hyperleptinemia, Ob-Rb may signal excessively through multiple signaling pathways, including JAK/STAT, insulin receptor substrate-2/phosphatidylinositol 3-kinase (IRS-2/ PI3K), and nitric oxide that may ultimately promote cardiovascular disease (CVD) through tissue-specific mechanisms[45].

INVOLVEMENT JAK/STAT IN OBESITY

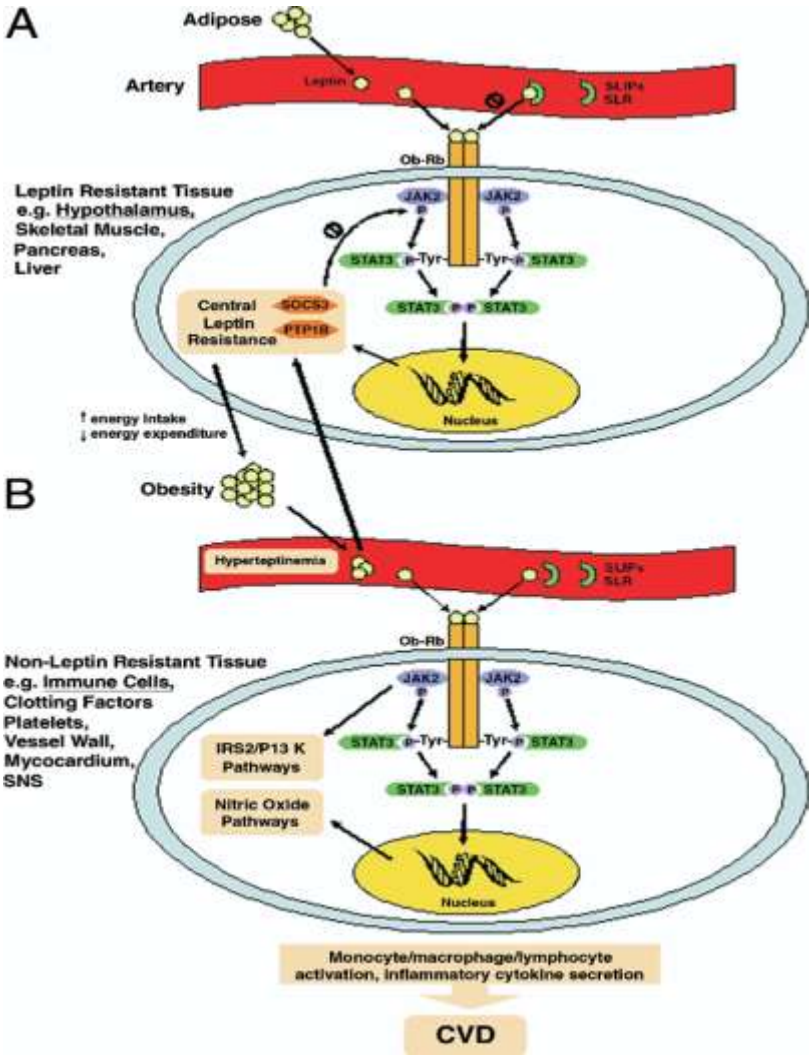

FIGURE 3

LUNG CANCER

Lung cancer, one of our research interests, causes more deaths *per* year than any other type of cancer in men and it is the second cause of death in women after breast cancer. The classification of human lung cancer includes two major types: small cell lung cancer (SCLC) and non-small cell lung cancer.

The main transcription factor induced by IL-6-type cytokines, such as IL-6, oncostatin M (OSM) and leukaemia inhibitory factor (LIF), is STAT3. IL-6 is a pleiotropic cytokine that plays important roles in cell proliferation, differentiation, survival and apoptosis. IL-6 also takes part in immune responses, haematopoiesis and inflammation (reviewed in . In addition, IL-6 can control a variety of responses in many cell types and is a crucial regulator of the nervous system, endocrine system, bone metabolism, amongst others . IL-6 induces transcription of the *IL-6* gene *via* JAK2 and STAT3.This is thought to lead to increased autocrine production of this cytokine observed in different cancer cell lines[46].

Thus, regulating JAK2 and/ or STAT3 could reduce IL-6 production, thereby impairing cell growth and enhancing their susceptiblity to other treatments [90]. Indeed, blockade of IL-6 signalling in lung cancerderived cell lines was shown to be enough to inhibit cell growth . It was also shown that some tumours in mice are induced by *ras*,which can also stimulate secretion of IL-6 in different cell types. *Ras*-induced transformation in a variety of mouse models appears to require IL-6 and, consistent with that, IL-6 knockout animals were more resistant to *ras*-induced carcinogenesis.Accordingly, knockdown, genetic ablation or antibody neutralization of IL-6 can limit tumour growth induced by *ras*. Interestingly, some human lung adenocarcinomas also have *RAS* mutated . Paradoxically, IL-6 can under certain circumstances decrease cell growth in some types of lung cancer cells . For example, the growth of Lewis lung cancer carcinoma cells decreased after being transfected with IL-6. When these cells were treated with an anti -IL-6 antibody they did not proliferate, indicating that growth inhibition was not related to a direct autocrine effect of IL-6. In contrast, in other NSCLC cell lines, IL-6 caused an increase in growth (A549, Calu3, Calu6, and H23). In the presence of IL-6 antisense phosphorothioated oligonucleotides, cell proliferation was notably reduced.

However, neither the presence of monoclonal neutralizing anti-IL-6 antibodies, nor exogenous IL-6, interfered with cell proliferation,or IL-6 synthesis. This probably reflects the now widely recognized importance of cellular background in determining cellular responses and biological outcome [22, 98]. Certainly in lung cancer patients, IL-6 appears to promote and sustain malignancy [47]. For example,IL-6 has been found elevated in lung cancer patients and the autocrine production of IL-6 has been shown to lead to constitutive activation of STAT3 and promote lung adenocarcinoma and malignant pleural infusion. Increased levels of circulating IL-6 thus appear to be an adverse prognostic factor for lung cancer patients [48].

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