JAK-STAT signaling: clinicopathology and therapeutics

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Questions?

- Outline the canonical steps in JAK-STAT signalling pathways?
- What is the clinical relevance of JAK2 mutations?
- List 5 disorders associated with mutations in the JAK-STAT signalling pathway?
Introduction

- Cellular responses to dozens of cytokines and growth factors are mediated by the evolutionarily conserved Janus Kinase-Signal Transducers and Activators of Transcription (JAK-STAT) pathway.

- JAK/STAT signalling is an elegant mechanism by which extracellular factors can control gene expression
  - Employed by cytokines, colony-stimulating factors and hormones
Introduction

• Responses include:
  - Proliferation
  - Differentiation
  - Migration
  - Apoptosis
  - Inflammation
  - Survival

• JAK-STAT signalling is essential for
  - Haematopoiesis
  - Immune cell development
  - Stem cell maintenance
  - Organism growth
Introduction

JAKs were identified through sequence comparisons as a unique class of tyrosine kinases that had both a
- Catalytic domain
- Kinase like domain

Hence the renaming from “just another kinase“ to an homage to the 2-faced roman god.

In mammals there are:
- 4 members of the JAK family
  - JAK1-3 and TYK2
- 7 STATs
  - STAT 1-4
  - STAT 5a, 5b
  - STAT6

Different JAK/STATs are recruited based on their tissue specificity and the receptors engaged in the signalling event.
<table>
<thead>
<tr>
<th>JAK/STAT</th>
<th>Important for signaling by</th>
<th>Knockout mouse phenotype</th>
<th>Genetic links to human disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK1</td>
<td>IFNα/β, IFN-γ, IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, IL-6 family cytokines, IL-10 family cytokines</td>
<td>Perinatally lethal</td>
<td>GOF somatic mutations cause ALL, AML, solid-organ malignancies</td>
</tr>
<tr>
<td>JAK2</td>
<td>IFNγ, IL-3, IL-5, GM-CSF, EPO, TPO, G-CSF, GH, leptin</td>
<td>Embryonically lethal due to absence of erythropoiesis</td>
<td>GOF mutations cause PV, PMF, ET, hypercoagulable state; somatic mutations associated with acute and chronic hematologic malignancies</td>
</tr>
<tr>
<td>JAK3</td>
<td>IL-2, IL-4, IL-7, IL-9, IL-15, IL-21</td>
<td>Defective T and B cell maturation</td>
<td>LOF mutation causes severe combined immunodeficiency (SCID)</td>
</tr>
<tr>
<td>TYK2</td>
<td>IFNα/β, IL-12, IL-23</td>
<td>Reduced responses to type 1 interferon and IL-12, and defective Stat3 activation</td>
<td>LOF mutation causes primary immunodeficiency</td>
</tr>
<tr>
<td>STAT1</td>
<td>All interferons</td>
<td>Impaired responses to type 1 and 2 interferon</td>
<td>LOF mutations confer susceptibility to mycobacterial and viral infections; GOF mutations cause chronic mucocutaneous candidiasis</td>
</tr>
<tr>
<td>STAT2</td>
<td>Type I IFNs</td>
<td>Impaired response to type 1 interferon and susceptibility to viral infections</td>
<td>Deficiency causes increased susceptibility to viral infections</td>
</tr>
<tr>
<td>STAT3</td>
<td>IL-6 and other gp130 cytokines</td>
<td>Embryonically lethal</td>
<td>LOF mutations cause AD-HIES; GOF somatic mutations strongly associated with LGL</td>
</tr>
<tr>
<td>STAT4</td>
<td>IL-12, IL-23, type 1 interferons</td>
<td>Mutations in mouse inhibit Th1 differentiation</td>
<td>Polymorphisms associated with RA, SLE</td>
</tr>
<tr>
<td>STAT5a/STAT5b</td>
<td>IL-2, EPO, TPO, GM-CSF, GH, IL-7</td>
<td>Defective hematopoiesis, other defects</td>
<td>Deficiency causes autoimmunity, bleeding diathesis, immunodeficiency, and dwarfism; somatic mutations associated with LGL</td>
</tr>
<tr>
<td>STAT6</td>
<td>IL-4, IL-13</td>
<td>Mutations in mouse inhibit T helper 2 differentiation</td>
<td>Polymorphisms associated with asthma, atopy, increased levels of IgE</td>
</tr>
</tbody>
</table>
Overview of JAK-STAT signaling

Figure 1
(1) When a cytokine engages its receptor, JAKs become activated and phosphorylate each other, as well as the intracellular tail of their receptors. (2) This creates a docking site for STATs, which are now able to bind to the cytoplasmic domain of the receptor. (3) The STATs, in turn, are phosphorylated and activated, which allows them to dimerize. (4) The STAT-STAT dimer translocates to the nucleus, where it can directly bind DNA and regulate gene expression.

JAK-STAT signaling steps

- Ligand binding
- Receptor oligomerisation
- 2 or more receptor associated JAKs are brought into close proximity
- JAK auto/trans phosphorylation
- Activation of JAK leads to conformational changes that distance their kinase domains from inhibitory pseudokinase domains.
- Jaks phosphorylate the signature cytoplasmic tyrosine residues to create docking site for STATs
- STATs bind JAK via SH2 domain
JAK-STAT signaling steps

• Phosphorylation of the conserved tyrosine residue between the SH2 domain and the C-terminus transactivation domain results in formation of STAT dimers.

• STAT dimers translocate to the nucleus via the nuclear pore complex, where they bind palindromic sequences within the promoter regions of target genes
  
  – In CD4+ T-cells thousands of STAT binding sites have been identified for each family member.
  – STATs are influenced by epigenetic changes and can also participate in them e.g. STAT4 and STAT6 each create open chromatin loci.
Non-canonical JAK-STAT signaling

- Pathways other than STATs are activated by cytokines
- Non-cytokine pathways can activate STATs
- Functions have been ascribed to unphosphorylated STATs
- STATs have non-nuclear functions e.g. STAT3 localises to the mitochondria
- JAKs may have actions in the nucleus independent of STATs
Non-redundant biological roles of JAK

- JAK1 KO mice die shortly after birth
  - Required for IL2,4,7,9,15,21 signalling
  - Required for Class II IFNa/b, gamma and IL-10 receptors

- JAK2 KO mice die in utero
  - Lack of erythropoiesis

- JAK3 mutation has been reported in SCID
  - Required for common γ-chain signalling
Non-redundant biological roles of STAT

- **STAT1** deficient mice have deficiencies in IFNa and IFNy responses
  - Susceptible to listeria and VSV
- **STAT2** deficient mice also sensitive to viral infections
- **STAT3** deficient mice die *in utero*
  - Due to a failure to form visceral endoderm and defects in lung, bone, colon, heart, CNS and skin
- **STAT4** deficient mice fail to respond to IL-12 and IL-23
  - Reduced Th1 and NK cell function
- **STAT5a and 5b** deficiency
  - Impaired growth hormone and prolactin function
  - Impaired mammary gland development
  - Growth retardation
  - Double KO die perinatally
- **STAT6** deficient mice
  - Refractory to IL-4 and IL-13; Defective Th2 polarisation and IgG1 and IgE class switching
  - Increased susceptibility to parasite infection
Non-redundancy of JAK-STAT in mice

Figure 1. Non-redundant JAK/STAT signalling in mice
Schematic showing the preferential cytokine/growth factor usage of different JAKs and STATs, as based on gene-targeting studies in mice. Emphasis in bold indicates the dominant JAK of the pair and colour coding links the individual cytokine/growth factors with their requisite STAT/s. See text for references.
Regulation of JAK-STAT

- Although the canonical JAK/STAT pathway is simple and direct, pathway components regulate or are regulated by members of other signalling pathways including:
  - ERK map kinase
  - PI3K
Negative regulation of JAK-STAT signaling

- A number of regulatory mechanisms have evolved to control the magnitude and duration of signalling

- 3 major mechanisms of control:
  - Receptor internalisation
    - Patients with mutations in the cytoplasmic tail of the G-CSF receptor have severe congenital neutropenia
    - Mutations block maturation signals and lead to defective ligand internalisation
    - **Predisposition to AML**
  - Dephosphorylation by phosphotyrosine phosphatases (PTPs)
    - CD45 in lymphocytes can dephosphorylate JAKs
  - Direct inhibition by protein inhibitors of STATs (PIAS) and suppressors of cytokine signalling (SOCS)
Suppressors of cytokine signaling (SOCS)

- Most studied JAK-STAT inhibitory proteins
- Family of small, cytokine inducible proteins that inhibit transduction by blocking JAK and STAT activation and phosphorylation
- Create a negative feedback loop
- 8 Family members
  - SOCS1-7 and CIS
- SOCS1 and SOCS3 can be recruited to the receptor complex by the SH2 domain, binding directly to the Jak or the receptor.
- SOCS1 deficient mice die shortly after birth due to widespread inflammation
- SOCS2 deficient mice have abnormal post-natal growth
- SOCS3 deficient mice die in utero
Specificity of JAK-STAT signaling

- How is JAK/STAT specificity achieved with so few building blocks?
- E.g. IL-6 and IL-10 are both potent STAT3 activators in myeloid cells but the former exerts mostly pro-inflammatory effects while the latter is mostly anti-inflammatory
- Specificity may be lineage dependent
Regulation of JAK-STAT

- Both STAT1 and STAT3 can be activated by JAK1
- In IL-6R signalling SOCS3 prevents IFNγ-like STAT1 transcriptional response
JAK in human disease

- Exaggerated or protracted JAK-STAT signalling has been implicated in a broad range of diseases.

- JAK3 LOF mutations:
  - X-linked SCID
    - Majority of patients lack common γ-chain required for IL-2,4,7,9,15,21 signalling
    - A subset of patients have JAK3 mutation
    - (JAK3 associates with γ-chain)
    - T-B+NK- (similar phenotype to common gamma chain)
JAK in human disease

- The majority of JAK2 mutations are GOF associated with
  - Acute leukemias
  - Polycythemia vera
  - Myeloproliferative disorders
- PV, ET and primary MF are closely related myeloproliferative disease characterised by increased BM production of erythrocytes and megakaryocytes.
  - The conditions may develop independently or simultaneously
  - There is a common genetic basis for these diseases in JAK 2 activating mutations
JAK in human disease

- These mutations may be chromosomal translocations, point mutations, insertions and deletions.

- Translocations can result in fusion of JAK2 catalytic kinase with multimerization subunits of partner proteins leading to constitutive tyrosine kinase activity and transformation.

- Many of the mutations are in the pseudo-kinase domain which has catalytic activity to limit auto activation of Jaks.
JAK2 in human disease

- JAK2 mutation in myeloproliferative diseases
- Frequently found in Brc-Abl negative MPD
  - 90% PV
  - 50% ET
  - 50% MF
- Mutated JAK2 may be able to bypass STAT control
- Interestingly, presence of this mutation in the general population is associated with increased mortality.

Figure 2. JAK and STAT domain organisation
Schematic showing the domain organisation of JAK and STAT proteins. The valine 617 commonly mutated in JAK2 in myeloproliferative neoplasms is shown. NT: N-terminal region, DBD: DNA binding domain, TAD: transcriptional activation domain.
JAK signaling as a therapeutic target

- JAKs have become attractive targets for novel therapeutics development for haematological and immunological disorders
  - “Jakinibs”
- Most development has focused in small molecule inhibitors of JAK2
- Ruxolitinib (JAK1 and JAK2)
  - First FDA approved Jakinib
  - Approved for RA
  - In trials for:
    - Psoriasis
    - IBD
    - Transplant rejection
    - JIA
- Tofacitinib (JAK3>2>1)
  - First selective Jakinib approved for human use
  - Approved for use in RA as monotherapy or with MTX
  - Non inferior to Adalimumab
  - Being trialled in psoriasis, psoriatic arthritis, JIA, transplant rejection
JAK signaling as a therapeutic target

- Oclacitinib approved for dermatitis in dogs
- Topical and oral Jakinibs are being trialled in immune-mediated alopecia
- TG101348 (JAK)
  - In phase II/III trials for myeloproliferative neoplasms
  - Patient demonstrate improvement in constitutional symptoms, reduction in spleen size
    - ?survival benefit
- Rates of infection with Jakinibs are higher than with placebo:
  - Common bacterial respiratory, genitourinary and GIT infections
  - PJP and MTB and CMV have also been reported
  - Patients on Tofacitinib are particularly at risk of HZV
  - May also develop neutropenia and anaemia
JAK signaling as a therapeutic target

JAKinibs: future directions

• 25 JAKinibs are being tested in a wide variety of conditions including:
  ➢ Asthma
  ➢ Malignancies
  ➢ MPD
  ➢ Autoimmune conditions
Baricitinib in Patients with Refractory Rheumatoid Arthritis

Mark C. Genovese, M.D., Joel Kremer, M.D., Omid Zamani, M.D., Charles Ludivico, M.D., Marek Krogulec, M.D., Li Xie, M.S., Scott D. Beattie, Ph.D., Alisa E. Koch, M.D., Tracy E. Cardillo, M.S., Terence P. Rooney, M.D., William L. Macias, M.D., Ph.D., Stephanie de Bono, M.D., Ph.D., Douglas E. Schlichting, M.S., and Josef S. Smolen, M.D.
HOT OF THE PRESS

- Dose-responsive improvement in ACR20 scores
- 2 NMSC and 2 serious CVD adverse events in higher dose group (1 fatal CVA)
STAT in human disease

• Hyper IgE Syndrome (HIES)
  ➢ STAT3 LOF
    • STAT 3 is crucial for the production of IL-17 important for host defence against staph aureus and fungal infections
  ➢ STAT1 GOF
  ➢ 1 report of TYK2 LOF

• Chronic Mucocutaneous Candidiasis (CMC)
  ➢ Persistent infection of skin, nails, mucosa with C.Albicans
  ➢ GOF STAT1 mutation causing exaggerated IFN-y signalling that inhibits IL-17 transcription

• IPEX Syndrome
  ➢ STAT1 GOF mutations have also been reported in cases with intact T-reg compartment
STATs in human disease

• Mycobacterial Infections
  - LOF STAT1 mutations are associated with recurrent mycobacterial infections and disseminated BCG.
  - STAT1 plays a known role in IFNγ signalling
STAT in human disease

• STAT1 mutations
  - LOF mycobacterial and viral infections
  - GOF fungal infections; CMC

• STAT2 mutations
  - One family has been described
  - Associated with viral infections

• STAT3 mutations:
  - LOF HIES, fungal infections; CMC
  - GOF autoimmune disease, T1DM
  - STAT3 SNPs associated with Crohns Disease, SLE, SJS

• STAT5B mutations develop autoimmunity due to a defect in T-reg

• STAT6 SNP associated with asthma
STAT signaling as a therapeutic target

- STATs do not have catalytic activity and therefore are more challenging as a therapeutic target
- Issues with bioavailability, in vivo efficacy and selectivity
- Targeting of STATs may be achieved by:
  - blocking phosphorylation
  - disrupting SH2 domains
  - interfering with DNA binding
    - Oligonucleotide-based STAT inhibitors are currently undergoing testing for malignancies
## JAK-STAT signalling: Novel therapeutic targets

### Table 2: Jakinibs and STAT inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Status</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruxolitinib (INC424)</td>
<td>JAK1, JAK2</td>
<td>FDA approved</td>
<td>Polycythemia, myelofibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>Various cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase 2b</td>
<td>Psoriasis (topical)</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>JAK3 &gt; JAK1 &gt; JAK2</td>
<td>FDA approved</td>
<td>RA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase III</td>
<td>Psoriasis, ulcerative colitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>Spondyloarthropathy, JIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transplant rejection</td>
</tr>
<tr>
<td>Oclacitinib</td>
<td>JAK1</td>
<td>FDA approved</td>
<td>Canine allergic dermatitis</td>
</tr>
<tr>
<td>Baricitinib</td>
<td>JAK1, JAK2</td>
<td>Phase II</td>
<td>RA, psoriasis, diabetic nephropathy,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>autoimmune inflammatory disease</td>
</tr>
<tr>
<td>Momelitinib</td>
<td>JAK1, JAK2</td>
<td>Phase III</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>GLPG0634</td>
<td>JAK1</td>
<td>Phase II</td>
<td>RA, Crohn's disease</td>
</tr>
<tr>
<td>INCB047986</td>
<td>JAK inhibitor</td>
<td>Phase I</td>
<td>Lymphoma, solid tumors</td>
</tr>
<tr>
<td>INCB039110</td>
<td>JAK1, JAK2</td>
<td>Phase II</td>
<td>Psoriasis, RA</td>
</tr>
<tr>
<td>CYT387</td>
<td>JAK1, JAK2</td>
<td>Phase II</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>ASP015K</td>
<td>JAK1 &gt; JAK3 &gt; JAK2</td>
<td>Phase II</td>
<td>Psoriasis, RA</td>
</tr>
<tr>
<td>R333</td>
<td>JAK/SYK</td>
<td>Phase II</td>
<td>Discoid lupus (topical)</td>
</tr>
<tr>
<td>PF-04065842</td>
<td>JAK1</td>
<td>Phase I</td>
<td>Healthy adults</td>
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<tr>
<td>GLG0778</td>
<td>JAK1</td>
<td>Phase II</td>
<td>SLE</td>
</tr>
<tr>
<td>GSK2586184</td>
<td>JAK1</td>
<td>Phase II</td>
<td>SLE, psoriasis</td>
</tr>
<tr>
<td>VX-509</td>
<td>JAK3</td>
<td>Phase 1b</td>
<td>RA</td>
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<tr>
<td>Lesauritinib</td>
<td>FLT3, JAK2, TRKs</td>
<td>Phase II</td>
<td>AML, PV/ET, myelofibrosis</td>
</tr>
<tr>
<td>Pacritinib</td>
<td>JAK2</td>
<td>Phase II</td>
<td>Myelofibrosis, myeloid leukemias, MDS</td>
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<tr>
<td>LY2784544</td>
<td>JAK2</td>
<td>Phase II</td>
<td>Myelofibrosis, various cancers</td>
</tr>
<tr>
<td>AZD1480</td>
<td>JAK1, JAK2</td>
<td>Phase I</td>
<td>Myeloproliferative diseases, various cancers</td>
</tr>
<tr>
<td>XL019</td>
<td>JAK2</td>
<td>Phase I, terminated</td>
<td>Myelofibrosis, PV</td>
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<tr>
<td>BMS-911543</td>
<td>JAK2</td>
<td>Phase II</td>
<td>Myelofibrosis</td>
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<td>NS-108</td>
<td>JAK2, SRC</td>
<td>Phase II</td>
<td>Myelofibrosis</td>
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<tr>
<td>PF-06263276</td>
<td>pan-JAK</td>
<td>Phase I</td>
<td>Healthy adults (topical)</td>
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<tr>
<td>SV1358</td>
<td>JAK2, Flt3</td>
<td>Phase I</td>
<td>Healthy adults</td>
</tr>
<tr>
<td>ISIS-STAT3Rx (AZD9150)</td>
<td>STAT3</td>
<td>Phase II</td>
<td>Various cancers</td>
</tr>
<tr>
<td>OP8-51602</td>
<td>STAT3</td>
<td>Phase I</td>
<td>Nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>OPB-31121</td>
<td>STAT3</td>
<td>Phase I</td>
<td>Various cancers</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; ET, essential thrombocythemia; JIA, juvenile idiopathic arthritis; PV, polycythemia vera; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.
JAK/STAT signaling and oncogenesis

- STAT hyperactivity can drive cellular transformation downstream of classic oncogenic signals like BCR-ABL, Ras and SRC.
- Typically involves STAT3 and STAT5 and is considered a defining characteristic of many solid and blood cancers.
  - STAT3 has been shown to limit anti-tumour immunity via limiting IL23 signalling which is pro-oncogenic.
- GOF JAK2 mutations underlie myeloproliferative malignancies
- Few mutant STAT alleles are associated with carcinogenesis suggesting their oncogenic potential is secondary to upstream events.
JAK-STAT signalling: Novel therapeutic targets

Conclusions

• The JAK-STAT Pathway is a central paradigm for understanding cellular signaling.

• Further understanding its role in health and disease may lead to insights into disease pathogenesis, diagnostic advances and therapeutic options.

• Understanding how to selectively inhibit components of JAK-STAT signaling remains a current area of research.

• Further work is required to elucidate exact mechanisms of Jakinib actions and to evaluate second generation inhibitors currently in development.
Answers to questions

• Outline the canonical steps in JAK-STAT signalling pathways?

Figure 1
(1) When a cytokine engages its receptor, JAKs become activated and phosphorylate each other, as well as the intracellular tail of their receptors.
(2) This creates a docking site for STATs, which are now able to bind to the cytoplasmic domain of the receptor.
(3) The STATs, in turn, are phosphorylated and activated, which allows them to dimerize.
(4) The STAT–STAT dimer translocates to the nucleus, where it can directly bind DNA and regulate gene expression.
What is the clinical relevance of JAK2 mutations?

JAK2 is a tyrosine kinase involved in signalling for a number of cytokines, growth factors and hormones.

These include IFNγ, EPO, and G-CSF, GM-CSF.

JAK2 KO mice die embryonically due to failure of erythropoiesis.

In humans JAK2 mutations are associated with a number of Bcr-Abl negative myeloproliferative disorders including:

- Polycythemia Vera
- Essential thrombocythemia
- Primary Myelofibrosis

The majority of JAK2 mutations are V617F in the pseudokinase kinase domain which normally acts to limit JAK2 phosphorylations.

- Mutations therefore lead to constitutive JAK2 activation
- Used in diagnosis of MPD

Jakinib small molecule inhibitors e.g. Ruxolitinib are approved for use in RA and in trials for MPD and other conditions.
### Answers to questions

- **List 5 disorders associated with mutations in the JAK-STAT signalling pathway?**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK3 LOF</td>
<td>X-linked SCID (T-B+NK-)</td>
</tr>
<tr>
<td>JAK2 GOF</td>
<td>MPD: PV, ET, MF</td>
</tr>
<tr>
<td>Tyk2 LOF</td>
<td>HIES</td>
</tr>
<tr>
<td>STAT1 GOF</td>
<td>CMC</td>
</tr>
<tr>
<td>STAT3 LOF</td>
<td>HIE, CMC</td>
</tr>
</tbody>
</table>
Thank You

- Questions?