STAT5-mediated self-renewal of normal hematopoietic and leukemic stem cells

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The level of transcription factor activity critically regulates cell fate decisions such as hematopoietic stem cell self-renewal and differentiation. The balance between hematopoietic stem cell self-renewal and differentiation needs to be tightly controlled, as a shift toward differentiation might exhaust the stem cell pool, while a shift toward self-renewal might mark the onset of leukemic transformation. A number of transcription factors have been proposed to be critically involved in governing stem cell fate and lineage commitment, such as Hox transcription factors, c-Myc, Notch1, β-catenin, C/ebpα, Pu.1 and STAT5. It is therefore no surprise that dysregulation of these transcription factors can also contribute to the development of leukemias. This review will discuss the role of STAT5 in both normal and leukemic hematopoietic stem cells as well as mechanisms by which STAT5 might contribute to the development of human leukemias.
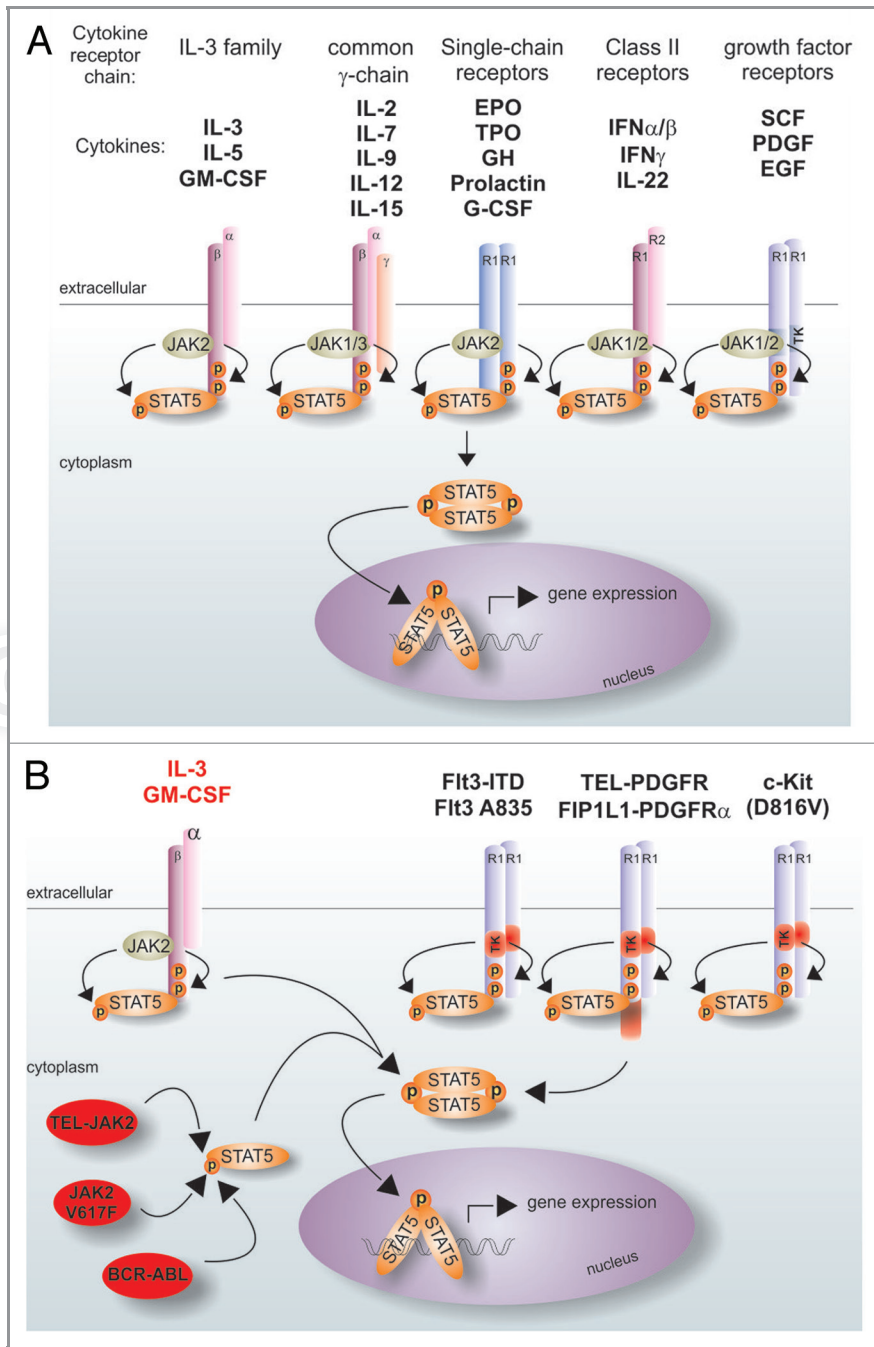
Introduction

Signal Transducer and Activator of Transcription 5 (STAT5) is widely expressed throughout the hematopoietic system, both in stem and progenitor cells as well as in committed erythroid, myeloid and lymphoid cells.[1-3] Indeed, it is not surprising that STAT5 can be activated by a wide variety of cytokines and growth factors.[3-5] These include cytokines and growth factors that can signal through the Interleukin 3 (IL3)-receptor family [IL3, IL5, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF)], through the common γ-chain receptor family (IL2, IL7, IL9, IL12, IL15), through single chain receptors [Erythropoietin (EPO), Thrombopoietin (TPO), Growth Hormone (GH), prolactin, Granulocyte-Colony Stimulating Factor (G-CSF)], through class II receptors [Interferon α (IFN-α), IFN-γ, IL22] or through tyrosine kinase receptors [Stem Cell Factor (SCF), Platelet Derived Growth Factor (PDGF), Epidermal growth Factor (EGF)] (**Fig. 1**). In most cases, Janus Kinase (JAK) tyrosine kinase activity mediates STAT5 tyrosine phosphorylation, and STAT5 can be activated by JAK1, 2 or 3, depending on the cytokine-activated receptor complex. Alternatively, the tyrosine kinase receptor family can also induce STAT5 phosphorylation in a JAK-independent manner. While STAT5 is expressed in the

majority of hematopoietic cell types, the cytokine receptor expression is much more tissue-specific. Thus, specific cytokines are able to induce STAT5 activity in subsets of cell types only.[6] For example, cytokines that activate STAT5 in the most immature human hematopoietic stem compartment include SCF[7] and TPO.[8] These cytokines have been shown to promote long-term hematopoiesis in vitro,[9] and hypersensitivity to TPO in Lnk^{−/−} mice resulted in elevated stem cell self-renewal, which coincided with increased levels of STAT5 activity.[10] Within the erythroid compartment, STAT5 is activated by EPO,[11] where STAT5 fulfills an important anti-apoptotic role by upregulating Bcl-X$_l$,[12-15] although a more direct role in initiating erythroid commitment might exist as well.[16-18] In myeloid cells, STAT5 can be activated by a variety of cytokines, including IL3, IL5, GM-CSF and CSF1 (reviewed in ref. 3). Although initially in STAT5AB[ΔN/ΔN] mice myelopoiesis appeared to be relatively unaffected,[19] it is likely that in myeloid cells many of the signals initiated by e.g., IL-3 and GM-CSF are, at least in part, mediated by STAT5,[20,21] thereby regulating myeloproliferation or anti-apoptosis.[22-24] During myelosuppression, mice completely deficient of STAT5AB failed to produce enhanced levels of neutrophils and were unable to respond to GM-CSF.[25] IL5-induced STAT5 activation is required for the induction of eosinophil differentiation.[26] Lymphoid development is severely impaired in STAT5^{−/−} mice.[27] STAT5 activation is required for IL2-induced T cell proliferation and the production of NK cells,[28,29] or for IL7-mediated B cell expansion.[30]

As summarized in **Table 1** and **Figure 1B**, a wide variety of genetic defects in myeloid leukemias and myeloproliferative diseases (MPDs) result in activation of the STAT5 pathway, including mutations in Flt3 and cKit receptors, JAK2 mutations, translocations such as TEL-PDGFRa, and Bcr-Abl, but also as a result of increased cytokine signaling. Numerous functional studies have indicated that aberrant activation of STAT5 can contribute to the process of leukemic transformation. Downstream of Flt3-ITD (Internal Tandem Duplication) mutations, STAT5 is strongly activated via two tyrosine residues within the Flt3 receptor, Y589 and Y592 that act as docking sites for the SH2 domain of STAT5 molecules.[31] Mutation of these residues into phenylalanines completely abrogated activation of STAT5, and importantly completely impaired induction of a myeloproliferative disease in vivo in a murine transplantation model.[31] Thus, it is likely that STAT5 signaling is essential for the transforming potential of Flt3-ITD.

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Figure 1. STAT5 signaling in normal and leukemic cells. (A) Normal cytokine-induced STAT5 signaling. (B) Constitutive STAT5 signaling in hematological malignancies.

expansion and the formation of leukemic CAFCs was strongly impaired by down-modulation of STAT5.[33] Although the presence of Flt3-ITDs was not the exclusive genetic mutation that induced constitutive STAT5 signaling in the samples that were studied, these data clearly underscore the important role that STAT5 fulfills in long-term expansion and self-renewal of primary AML stem/progenitor cells as well.

In chronic myeloid leukemia (CML) induced by Bcr-Abl, it has been convincingly demonstrated that STAT5 also plays an important role. A number of studies have shown that STAT5 is efficiently activated downstream of Bcr-Abl,[59-62] and interference with STAT5 activation negatively impacts the survival and proliferation of Bcr-Abl-expressing cells.[27,55,63-69] Bcr-Abl p210-transduced STAT5$^{\Delta N/\Delta N}$ murine bone marrow (BM) cells developed CML with low frequencies, with a delayed onset of disease.[55] The induction of acute lymphoid leukemia (ALL) was not impaired in these animals. Complete abrogation of STAT5 expression in STAT5AB$^{-/-}$ mice also impaired lymphoid transformation induced by Bcr-Abl-expressing murine BM.[27,70] In primary human CML cells, it was demonstrated that downmodulation of STAT5 expression by RNAi impaired Bcr-Abl-dependent proliferation and also reduced colony formation in methylcellulose.[68] Inhibition of STAT5 by pimozide reduced colony formation of CML CD34$^+$ cells, also in tyrosine kinase-resistant patient samples.[71]

In myeloproliferative diseases it has been demonstrated in mouse models that bone marrow (BM)-transduced with TEL-JAK2 no longer induced disease in recipient mice when the oncogene was introduced in a STAT5$^{\Delta N/\Delta N}$ background.[40] Similarly, MPD induced by TEL-PDGFRα depended on STAT5 activity.[50,51] Finally, enhanced STAT5 activity has been observed in Polycythemia Vera (PV), caused by the activating JAK2 V617F mutation.[72] Inhibition of JAK2 kinase activity abrogated the activation of STAT5, which coincided with a suppression of erythropoiesis in vitro and in vivo.[73,74]

The most direct evidence for STAT5 acting as an oncogene arises from murine BM transplantation studies in which constitutively activated STAT5 (S711F) mutants were overexpressed. Lethally irradiated recipients receiving activated STAT5-transduced BM died within 6 weeks after transplantation of a multilineage leukemia.[75] It was demonstrated that a tryptophan residue in the N-terminal region of STAT5 is required for

Although in human cells introduction of Flt3-ITD did not result in a myeloproliferative disease in transplanted NOD-SCID mice, the activated stem cell phenotype imposed on CB CD34$^+$ cells, as revealed by the formation of early cobblestone area forming cells (CAFCs), was impaired by coexpression of a dominant negative STAT5A (Y694F) mutant,[32] suggesting that also in human cells STAT5 is an important mediator of Flt3-ITD-induced signaling. In studies in which STAT5 expression was targeted in primary acute myeloid leukemia (AML) CD34$^+$ cells using a lentiviral approach, it was observed that long-term

Table 1. STAT5 activation in hematological malignancies

Kinase	Mutation/Translocation	Disease	References
Flt3	ITD (exon 11–12, 3–400 bp)	AML	31, 34–38
	TK point mutations (Asp835)	AML	39
JAK2	TEL-JAK2	ALL, CML, MPD	40
	V617F	PV, ET, IMF	41–44
JAK3	A572V, V722I, P132T	AMKL	45
c-Kit	D816V	AML	46–48
	ITD (exon 11–12)	Pediatric AML	49
PDGFR	TEL-PDGFR	CML, MPD	50, 51
	FIP1L1-PDGFRα	Chronic Eosinophilic Leukemia	52, 53
Abl	v-ABL (p160)	CML, BCL	54
	BCR-ABL (p210, p185)	CML	27, 55
FGFR	ZNF198-FGFR1	MPD	56
RARα	STAT5b-RARα	APL	57, 58

tetramerization of STAT5 dimers, and tetramer-deficient STAT5 mutants were unable to induce leukemia in mice.[75] Another activating mutant of STAT5, STAT5A(1*6) that contains two point mutations (H299R and S711F)[76] was earlier shown to induce myeloid hyperproliferation, but not leukemia, in a murine retroviral overexpression model.[40] This was later confirmed by others, and a fatal MPD was observed by overexpression of these STAT5 mutants, but only when the most primitive CD34⁻Lin⁻cKit⁺Sca1⁺ (LSK) population was transduced and used for transplantation to irradiated recipients, suggesting that the stem cell, but not a committed progenitor is the target cell for transformation induced by activated STAT5.[77] Intriguingly, while these examples clearly demonstrate that STAT5 can transform murine hematopoietic stem cells (HSCs), no in vivo STAT5-induced transformation has been reported in human cell populations. While enhanced self-renewal and long-term stem cell maintenance can be achieved by introduction of activated STAT5 in human CD34⁺ cells, a myeloproliferative disease or leukemia does not occur in non-obese diabetic/severe combined immuno-deficiency (NOD-SCID) transplantations models.[16] It is plausible that the NOD-SCID xenograft model is not suitable to completely recapitulate human disease, or alternatively it is possible that species-specific differences in STAT5 signaling exist. In line with these observations, introduction of Bcr-Abl in murine BM resulted in a rapid and lethal MPD whereby recipients die within 3 weeks after transplantation. Introduction of Bcr-Abl in human CD34⁺ cells does not result in a rapid leukemia or MPD in engrafted NOD-SCID mice, and only after 5 mo progression to an early stage disease was observed in some animals.[78,79]

Collectively, these data indicate that STAT5 is frequently activated in various hematological malignancies, whereby it strongly affects processes such as self-renewal and lineage fate determination. Whether STAT5 target genes in normal and leukemic stem cells are identical, or whether leukemic stem cell-specific STAT5 target genes exist remains to be determined. Also, it will be informative to study how STAT5 might cooperate with additional leukemic oncogenes in a multi-hit approach to model the development of human leukemias.

STAT5 as a Stem Cell Self-Renewal Factor

Loss-of-function and gain-of-function experiments have revealed critical roles for STAT5 in the hematopoietic stem/progenitor compartment. STAT5AB^{ΔN/ΔN} mice have been used to assess stem cell function in the absence of wt STAT5 signaling. These mice were characterized by normal HSC numbers and stem cells isolated from the bone marrow or fetal liver were capable of engrafting irradiated recipients.[80] Yet, competitive repopulating capacity of STAT5AB^{ΔN/ΔN} HSCs was severely impaired.[80-85] The underlying mechanisms are not fully elucidated yet, but it has been observed that the responsiveness of STAT5AB^{ΔN/ΔN} HSCs to early-acting cytokines such as IL3 and SCF was reduced, while the sensitivity to 5-fluoroacil was enhanced.[81] Loss of protection against apoptosis most likely does not explain the STAT5AB^{ΔN/ΔN} HSCs phenotypes, as overexpression of Bcl2 was not sufficient to rescue repopulating defects.[84] Although homing of STAT5AB^{ΔN/ΔN} BM cells into lethally irradiated recipients was not impaired,[83] retention in the bone marrow was reduced under non-myeloablative conditions,[86] leaving open the possibility that competition for the niche might play a role. Mice completely devoid of STAT5 display severely impaired hematopoiesis.[87] More recently, using an Mx1-Cre inducible mouse model, it was shown that conditional deletion of STAT5 results in a loss of stem cell quiescence, associated with reduced survival and gradual loss of the HSC pool.[88]

In order to study STAT5 signaling in human hematopoietic stem/progenitor cells, we have used a lentiviral shRNA approach in cord blood (CB) CD34⁺ cells.[33] Downmodulation of STAT5 to about 30% of the endogenous levels reduced progenitor frequencies as determined by Colony Forming Cell (CFC) assays in methylcellulose as well as stem cell frequencies as determined by Long-Term Culture-Initiating Cell (LTC-IC) assays in limiting dilution.[33] This resulted in reduced long-term expansion on MS5 bone marrow stroma upon downmodulation of STAT5 expression, whereby the myeloid and erythroid differentiation were unaffected. Single-cell assays using transduced CD34⁺/CD38⁻ cells revealed that cell cycle progression induced by early-acting cytokines SCF and TPO was impaired by STAT5 downmodulation.[33]

Reversely, activating mutants of STAT5 have been introduced in murine CD34⁻LSK cells and the effects on stem and progenitor cells were assessed in vitro and in vivo.[77] Introduction of STAT5A(1*6) or STAT5A(1*7) mutants resulted in a strong ex vivo expansion of immature CFU-nmEM progenitors, without affecting the symmetry of stem cell divisions as determined in paired-daughter cell assays.[77] Importantly, long-term repopulating HSCs could be maintained under ex vivo culture conditions as CD34⁻LSK cells expressing activated STAT5 had a strong competitive repopulating advantage over wild type cells after 7 d and 10 d ex vivo culturing in the presence of SCF or SCF and TPO.[77]

Activating mutants of STAT5 have also been introduced in human CD34⁺ cells. Overexpression of STAT5A(1*6) in human CD34⁺ cord blood cells resulted in enhanced stem cell self-renewal.[16] This enhanced self-renewal was only observed in bone marrow stromal cocultures, but not in cytokine-driven liquid

culture conditions. These data argued that STAT5-induced HSC cell self-renewal depends on the presence of a bone marrow microenvironment, and it was indeed observed that STAT5A (1*6)-expressing CD34+ cells have a strongly enhanced interaction with bone marrow stromal cells, resulting in the appearance of early CAFCs underneath the stroma within 1 week after plating. These CAFCs contained self-renewal potential as demonstrated by their capacity to give rise to second CAFCs upon harvest and replating onto new stroma, as well as by their capacity to engraft in sublethally irradiated NOD-SCID mice. Upon serial replating, long-term cultures could be established by over-expression of activated STAT5 for over 20 weeks, giving rise to new CAFCs upon each replating as well as to progeny in suspension. Hematopoietic progenitors could be maintained long-term in these culture conditions and the suspension cells retained an immature blast-like morphology.[16]

When STAT5A(1*6) mutants were expressed in murine embryonic stem (ES) cells, the generation of hematopoietic stem cells was greatly facilitated as studied on OP9 bone marrow stromal cells.[17] The generation of hematopoietic CAFCs was strongly enhanced by activated STAT5. Importantly, these CAFCs could be serially passaged onto new OP9 stroma, giving rise to second and third CAFCs that were able to sustain long-term hematopoiesis and generate high numbers hematopoietic progenitors, indicative of HSC self-renewal in vitro. Also, the CAFCs generated by activation of STAT5 could engraft sublethally irradiated NOD-SCID mice, indicating that STAT5 facilitates the generation of ES-derived HSCs that can contribute to hematopoiesis in vivo as well.[17]

Mechanisms Involved in STAT5-Induced HSC Self-Renewal

Although various STAT5 target genes have been identified, the mechanisms by which STAT5 acts on HSCs remain to be elucidated. Using cell lines or heterogeneous stem/progenitor cell populations, enhanced cell growth is one of the most dominant phenotypes that is frequently observed in various studies, and several genes that are regulated by STAT5 associate with cell proliferation and cell cycle progression, including Cyclin D1, Pim1 and c-Myc.[16,89-91] CyclinD1$^{-/-}$D2$^{-/-}$ mice exhibit defects in the expansion of hematopoietic stem/progenitor cells.[92] Pim serine/threonine kinases act as mediators of cytokine-induced cell growth[93] by promoting acceleration of cell-cycle progression both at the G1/S and G2/M transitions by phosphorylating and activating the phosphatases Cdc25A and Cdc25C, respectively.[94,95] c-Myc controls the balance between self-renewal and differentiation of HSCs.[96,97] However, when more purified stem cell populations were studied using a conditional deletion Mx1-Cre model it was observed that STAT5 was required to maintain HSC quiescence. Upon STAT5 deletion a decrease in the percentage of cells in G0 within the long-term and short-term HSC compartments was observed, coinciding with a decrease in expression of quiescence-associated genes such as p57 and Tie2.[88] Loss of STAT5 also coincided with an increase in AnnexinV+ CD34−LSK cells.[88] In erythroid cells, it has been convincingly demonstrated that STAT5 contributes to cell survival by upregulating the anti-apoptosis gene Bcl-X$_L$.[13,14] Survival of Flt3-ITD+ AML cells has been shown to depend on STAT5-mediated expression of Mcl1.[98] Also, Bcl2 can be upregulated by STAT5 and is required to prevent apoptosis during terminal differentiation of myeloid cells.[23] Whether prevention of apoptosis contributes to STAT5-induced HSC self-renewal is currently unclear, but Bcl2 overexpression was not sufficient to rescue the repopulation defects of STAT5AB$^{\Delta N/\Delta N}$ HSCs,[84] suggesting that protection against apoptosis is not the main role of STAT5 signaling in HSCs. In our co-cultures, despite strong reductions in LTC-IC and CFC frequencies, we also did not detect an increased rate of apoptosis in STAT5 RNAi-transduced CD34+ CB cells, and no decreased expression of the Bcl-X$_L$ gene was observed.[33] The basic helix-loop-helix transcriptional inhibitor ID1 is also upregulated by STAT5,[99] and ID1-deficient HSCs fail to self-renew, leading to low steady-state HSC numbers and premature HSC exhaustion.[100] Little evidence exists that STAT5 affects the expression of other known HSC self-renewal regulators such as Bmi1 or HoxB4. Recently, we observed that STAT5 binds to and activates the promoter of Hypoxia Induced Factor 2α (Hif2α) in human CD34+/CD38− HSCs.[101] Functional studies indicated that STAT5-induced long-term expansion and elevated LTC-IC and CFC frequencies were reduced upon downmodulation of Hif2α.[101] Glucose uptake was enhanced in cells expression activated STAT5, coinciding with a Hif2α-dependent upregulation glucose metabolism genes, suggesting that pathways normally active under hypoxia might be utilized by STAT5 under normoxic conditions as well to maintain stem cell properties.

The phenotype imposed on cells by STAT5 might well depend on the actual level of STAT5 activity that is induced. Using a 4-hydroxytamoxifen inducible system that allowed titration of STAT5 activity in human stem and progenitor cells we have demonstrated that the stem cell maintenance properties require intermediate STAT5 activation.[102] On the other hand, high STAT5 activation levels resulted in erythroid differentiation at the expense of HSC self-renewal.[102] C/ebpα levels were reduced upon STAT5 activation, which reached maximum reduction levels at intermediate STAT5 activation. In line with these observations, in mice C/ebpα deficiency resulted in hyperproliferation of hematopoietic progenitor cells and enhancement of hematopoietic stem cell repopulating capacity and self-renewal.[103,104] Reintroduction of C/ebpα in STAT5A(1*6)-transduced human CD34+ cells was sufficient to impair HSC self-renewal capacity.[105] The mechanisms by which STAT5 affects C/ebpα expression levels are still under investigation, but these observations leave open the possibility that enhanced HSC self-renewal might in part be explained by reduction in C/ebpα expression levels.

It is remarkable that the effects of STAT5 on HSC self-renewal are confined to intermediate STAT5 activation levels.[102] This dosage effect of STAT5 on self-renewal is consistent with the observed constitutive activation of STAT5 in AML samples, which is typically lower then cytokine-induced STAT5 activation.[37,106,107] Such a dosage effect of transcription factors is at present not well understood, but besides STAT5 this has also been observed for the myeloid transcription factor Pu.1, which

at 20% expression gives rise to self-renewing murine myeloid leukemias, whereas 50% or 100% reduction in expression do not have such a dramatic effect.[108,109] Recently, also for Wnt signaling it was demonstrated that intermediate activation levels enhance self-renewal of HSCs.[110] Collectively, these examples clearly underscore the role of transcription factor dosage in regulating HSC self-renewal.

Cell Type-Specific STAT5 Signaling: Differential Role of STAT5 in Hematopoietic Stem and Progenitor Cells?

The observation that STAT5 drives cell cycle progression in various cell types and anti-apoptosis in others, while STAT5 is also required to maintain quiescence of hematopoietic stem cells, suggests that the cell-biological consequences of STAT5 signaling might be highly cell type-specific. We have addressed this issue by introducing a 4-hydroxytamoxifen (4OHT)-inducible STAT5-ER fusion in human stem and progenitor cells.[101] Activation of STAT5 specifically in HSC, common myeloid (CMP), granulo-cyte-macrophage (GMP) or megakaryocyte-erythroid progenitor (MEP) populations resulted in rather distinct phenotypes. Long-term self-renewal and enhanced cobblestone formation could

only be imposed on HSCs, but not on committed progenitor subpopulations. Erythroid differentiation could be induced in HSC, CMP and MEP populations, but not in GMPs. Gene expression profiling revealed that rather distinct gene expression profiles were induced in HSC as compared with more committed progenitor subpopulations. For instance, Tubb1, Hif2α, Sod2, IL8 and also the cell cycle inhibitor Cdkn1a/p21 were particularly upregulated in HSCs but not in committed progenitors (**Fig. 2**). In contrast, Osm, Pim1 and the negative feedback regulators CISH and Socs2 were upregulated both in HSCs and MPPs. The underlying mechanisms are currently unclear, but a number of possibilities might be hypothesized. First, it has been shown that several cofactors such as p300/Cbp, but also interactions with other transcription factors such as Foxo3a, can modulate and fine-tune the STAT5 response.[111-113] Cell type-specific interaction with such cofactors would then dictate a cell type-specific STAT5 activation pattern of target genes. Seen from this perspective, the modulation of STAT5 signaling by p300/Cbp could add to a stem vs. progenitor-specific component of STAT5. It has been observed that, unlike p300, Cbp is essential for HSC self-renewal maintenance, while p300 is suggested to play a role in differentiation.[114-116] Interaction of various transcription factors with p300/Cbp is facilitated by the p300/Cbp interacting protein
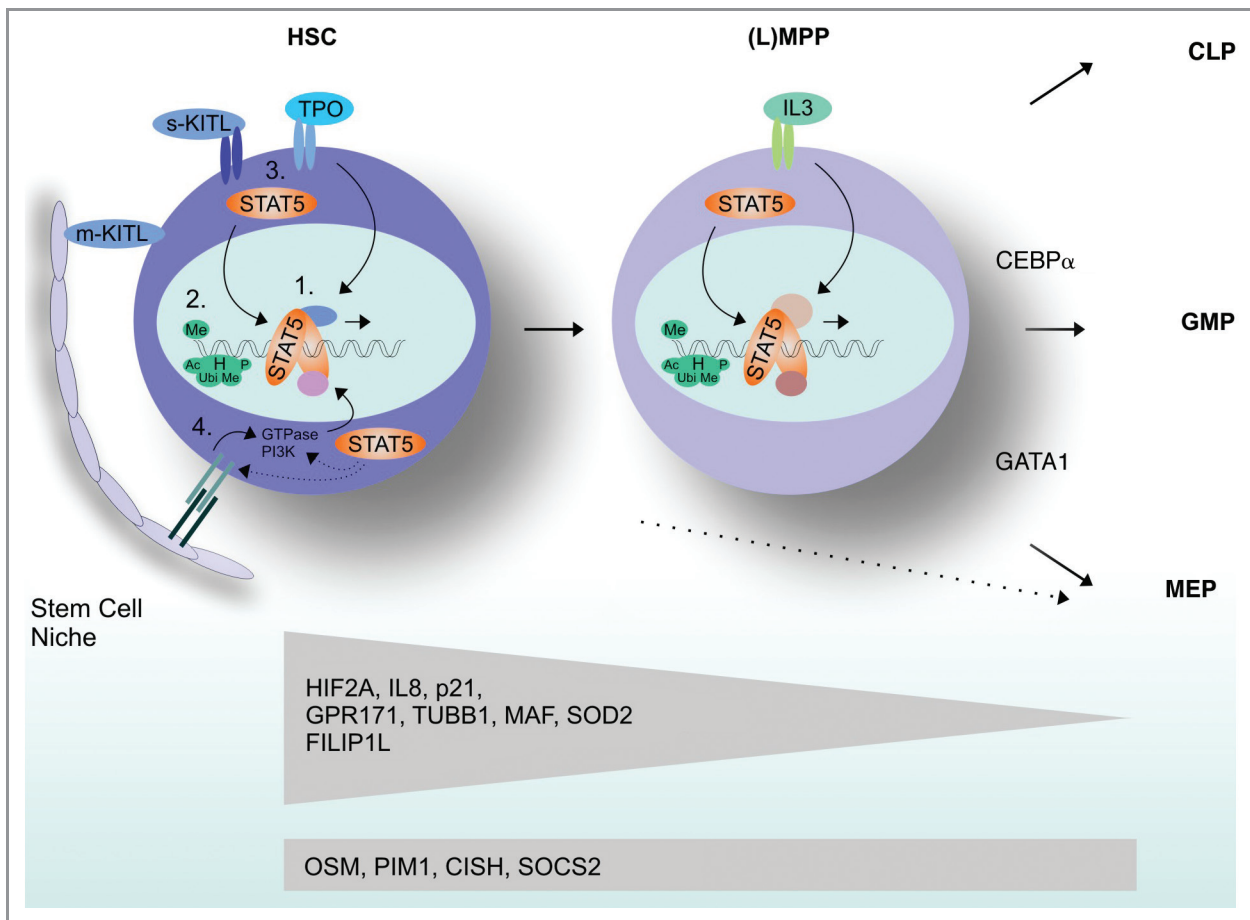
Figure 2. Cell type-specific STAT5 signaling in hematopoietic stem and progenitor cells. (1) Expression/activation of cofactors: complex composition. (2) Epigenetic factors that influence STAT5 DNA binding. (3) Expression of receptors and ligands. (4) Niche interactions.

Cited2 which has been shown to be a target gene of STAT5[113] and has differential expression and functions in hematopoietic stem vs. progenitor cells[117] (and our own observations). In part, such interactions can be mediated by posttranslational modifications such as serine phosphorylation or glycosylation of STAT5.[112] Thus, besides the induction of STAT5 tyrosine phosphorylation required for dimerization, nuclear translocation and DNA binding, the simultaneous activation of pathways that mediate STAT5 serine phosphorylation or glycosylation would be required.

Furthermore, the epigenetic status of the cell might play an important role. Hypermethylation of specific promoters or polycomb-mediated condensation of chromatin might prevent STAT5 association with regulatory promoter elements and thus transactivation of certain genes. Clearly, such differences in epigenetic status and cofactor expression might also be dictated by different responses to extracellular stimuli. Thus, the repertoire of specific cytokine and growth factor receptors that is expressed on a cell, as well as direct interactions between hematopoietic stem cells and their bone marrow niche, might ultimately determine the specific STAT5 response (**Fig. 2**).

Single cell tyrosine phospho-STAT5 analysis revealed that within the normal hematopoietic stem cell and progenitor compartment highly distinct cytokine-induced STAT5 activation patterns are observed.[6] Also in primary AML patient samples, rather heterogeneous responses toward a series of cytokines were observed, not directly linked to whether or not the cognate receptor was expressesed.[6] There was clear heterogeneity between different patient samples, but also different responses could be observed within distinct cellular compartments within a single patient.[6,118] For instance, in some patient samples strong IL3 and GM-CSF responses were observed, but only in the CD34⁻ subpopulation, while in other cases strong TPO responses were observed within CD34⁺/CD38⁻ and CD34⁺/CD38⁺ compartments. These observations clearly indicate that strong differences exist in how cytokine and growth factor signals are mediated within a certain cell type, both normal as well as leukemic.

Although elucidation of molecular mechanisms by which cell type specific STAT5 signaling is orchestrated needs further studies, cell type-specific STAT5 target genes clearly do exist. The observation that p21 is upregulated by STAT5, particularly in HSCs, is remarkable (our unpublished observations and ref. 101). It will be interesting to analyze whether the enhanced long-term self-renewal that is observed upon activation of STAT5 in hematopoietic stem cells[16] involves improved stem cell maintenance by keeping the HSCs pool in a relatively quiescent state via upregulation of p21. Knockout studies in mice have indicated that p21 is required during stress hematopoiesis,[119] and although p21 was also initially downregulated in STAT5 depleted LSK cells, this downmodulation was not maintained.[88] On the other hand, in murine embryonic fibroblasts it has also been shown that STAT5 can negatively regulate cell cycle progression through activation of p21.[120] Inhibition of JAK2/STAT5 signaling by the specific Jak2 inhibitor AZ960 stimulated cell cycling in CD34⁺/CD38⁻ cells in conjunction with downregulation of p21.[118] Further, activation of p21 has been shown to be critical in preventing excess DNA-damage accumulation and functional exhaustion of leukemic stem cells,[121] and it will be interesting to further reveal its role downstream of STAT5 in HSCs.

Furthermore, HIF2α was upregulated in HSCs and CMPs by STAT5, but not in MEPs and GMPs.[101] Under normoxic conditions, proline residues of Hypoxia-Induced Factor 2 are hydroxylated resulting in a reduction in protein levels via VHL-mediated proteasomal degradation. Under hypoxic conditions, such as in the presumed endosteal quiescent stem cell niche, Hifs are stabilized and act as transcription factors.[122] It is currently unknown whether and which Hif-induced target genes are essential to maintain stemness of normal HSCs, but it was recently shown that in Hif1⁻/⁻ mice HSCs numbers decrease during stress which was associated with a loss of HSC quiescence.[123] Another report indicated that HSCs in the quiescence niche utilize glycolysis for their energy demands, which depended on a Meis1-induced Hif1α signaling network.[124] Whether Hif1α and Hif2α display similar or distinct functions in HSCs remains to be established.

Our understanding of the mechanisms that determine whether, where and when a stem cell will self-renew or differentiate is still limited, but recent advances have indicated that the stem cell microenvironment provides essential cues that direct these cell fate decisions.[125-128] It is remarkable that STAT5-induced long-term self-renewal is typically observed when cells are cultured in direct contact with stromal cells,[16] in contrast to, e.g., Bmi1-induced self-renewal, which occurred in a more microenvironment-independent manner.[129] Thus, altered interactions with the stem cells niche might also underlie the enhanced self-renewal properties imposed on HSCs by activated STAT5. Although the mechanisms by which the interaction with the microenvironment of STAT5A(1*6)-expressing CD34⁺ cells are still unclear, our ongoing studies in which gene-expression profiling was performed in HSCs and progenitor subsets revealed that the list of STAT5-targets is significantly enriched for membrane (associated) proteins.[102] One of the STAT5 targets that has been identified is MUC1[130] which is a (proto)oncogene involved in adhesion and transendothelial migration, and has been associated with initiation of various intracellular signal transduction pathways including β-catenin, p53 and NFκB pathways.[131-136] Also, MUC1 has been shown to mediate an oscillatory calcium signal upon binding to ICAM1.[137] Within the endosteal region of the bone marrow where stem cells are thought to reside, Ca²⁺ levels are high, and HSC retention within the niche depends on the Calcium-Sensing receptor (CaR).[138] Thus, STAT5 might exert its phenotype, at least in part, by influencing interactions between HSCs and their niche.

Conclusions and Future Perspectives

In both murine and human model systems it has been convincingly shown that STAT5 fulfills an important role in hematopoietic stem cell self-renewal. Although the precise mechanisms by which HSC self-renewal is orchestrated by STAT5 remain elusive to date, an increasing number of STAT5 target genes have been identified that are currently under investigation. In myeloproliferative diseases and leukemias, a

number of oncogenes have been identified that are capable of inducing STAT5 activity, and accumulating evidence has indicated that STAT5 participates in self-renewal of leukemic stem cells as well. Thus, it appears likely that STAT5 will become an important diagnostic marker in the near future, and specific targeting of STAT5 should be focus of therapeutical intervention strategies to improve treatment of hematological malignancies.

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