STAT3 mutations identified in human hematologic neoplasms induce myeloid malignancies in a mouse bone marrow transplantation model

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ABSTRACT

STAT3 protein phosphorylation is a frequent event in various hematologic malignancies and solid tumors. Acquired *STAT3* mutations have been recently identified in 40% of patients with T-cell large granular lymphocytic leukemia, a rare T-cell disorder. In this study, we investigated the mutational status of *STAT3* in a large series of patients with lymphoid and myeloid diseases. *STAT3* mutations were identified in 1.6% (4 of 258) of patients with T-cell neoplasms, in 2.5% (2 of 79) of patients with diffuse large B-cell lymphoma but in no other B-cell lymphoma patients (0 of 104) or patients with myeloid malignancies (0 of 96). Functional *in vitro* assays indicated that the *STAT3Y640F* mutation leads to a constitutive phosphorylation of the protein. STA21, a STAT3 small molecule inhibitor, inhibited the proliferation of two distinct *STAT3* mutated cell lines. Using a mouse bone marrow transplantation assay, we observed that *STAT3Y640F* expression leads to the development of myeloproliferative neoplasms with expansion of either myeloid cells or megakaryocytes. Together, these data indicate that the *STAT3Y640F* mutation leads to constitutive activation of STAT3, induces malignant hematopoiesis *in vivo*, and may represent a novel therapeutic target in some lymphoid malignancies.

Introduction

STAT transcription factors are key regulators of gene transcription activated by cytokine receptors and JAK signaling, and are involved in numerous cellular processes, including hematopoietic differentiation, proliferation and immune responses. Aberrant activation of STAT factors, including STAT3, is frequently observed in hematologic malignancies and solid tumors. It is generally thought that aberrant STAT activation results from mutation or constitutive activation of upstream members of the signaling pathway, like in myeloproliferative neoplasms (MPN) in which *JAK2* and *MPL* genes are frequently mutated activating several STAT factors including STAT3.[1,2] Somatic mutations of *STAT3* associated with a constitutive phosphorylation, dimerization and activation of STAT3 have been identified in 6% of human inflammatory hepatocellular adenomas[3] and may be a hallmark of T-cell large granular lymphocytic leukemia (TGLL).[4] In a number of hematologic malignancies, the mechanism of constitutive STAT3 activation remains unexplained.[5]

Methods

Patients

Lymph node and peripheral blood samples from the patients were obtained with their informed consent and the approval of the local research ethics committees (Centre Henri Becquerel, Pitié-Salpétrière, Cochin, Bordeaux and Toulouse hospitals). Diagnoses were made by standard international criteria.

Polymerase chain reaction and DNA sequencing.

Polymerase chain reaction (PCR) primers (*Online Supplementary Table S1*) were designed to amplify and sequence 5 coding exons of *STAT3* (3, 6, 17, 21, 22). Nucleotide sequences were compared to wild-type human genomic sequence present in the databases (genome.ucsc.edu). All observed *STAT3* mutations were detected by bidirectional sequencing.

RNA-seq

RNA-seq was performed on an Illumina HiSeq 2000 using paired-end sequencing of 150–250-bp inserts and 100-bp reads (Fasteris).

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Constructs

Full-length WT and mutant Y640F *STAT3* open reading frame were cloned from a pCMV6-XL4 vector into an MSCV240-IRES-GFP retroviral vector.[3]

Cell culture

Ba/F3, YT1, FEPD and K562 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS). For growth of Ba/F3, medium was supplemented with 10 ng/mL mouse interleukin 3 (IL-3). For infections, Ba/F3 were mixed with viral supernatants obtained following standard procedures, spinfected for 90 min at 1800 g, and returned to the incubator. Forty-eight hours after infection, cells were sorted for GFP expression. For pharmacological treatment, cells were cultured in regular media with STA-21, a small STAT3 molecule inhibitor (25 μM), for 72 h. The number of viable cells was assessed by counts every day using the trypan blue exclusion method.

Western blot analysis and immunoprecipitation

Western blot (WB) and immunoprecipitation were performed following standard procedures and using 20 x 10^6 cells for each point. Eluates obtained after immunoprecipitation were analyzed by Western blot for STAT3 C20 (Santa Cruz) and phospho-STAT3 Tyr705 (Cell Signaling).

Flow cytometry

For phospho-specific flow cytometry, cells were fixed in methanol-free formaldehyde 1% for 10 min, washed with PBS, permeabilized with ice-cold 100% methanol for 30 min, and saturated with 0.5% BSA overnight. Total white blood cells and single-cell suspensions from bone marrow and spleen were stained *in toto* in PBS supplemented with 2% FBS with fluorochrome-conjugated mouse antibodies (*Online Supplementary Table S2*) raised against specific hematopoietic lineages markers.

Animal experiments

Wild-type (WT) C57BL/6 donor mice were injected with 5-fluorouracile (5-FU) five days prior to bone marrow (BM) collection. On Day 0, primary BM cells were obtained from femurs and tibiae, subjected to red blood cell lysis buffer and cultured overnight in RPMI 1640 supplemented with 10% FBS+IL-3, IL-6, and SCF (10 ng/mL each). On Day 1 and Day 2, cells were mixed with identical titer viral supernatants and spinfected for 90 min at 1800 g. After the second spinfection, 1 × 10^6 cells were injected in the tail veins of lethally irradiated C57BL/6 recipients. After transplantation, animals were monitored daily for signs and symptoms of disease and monthly by blood counts. The percentages of GFP-positive cells at various time points after transduction are indicated in *Online Supplementary Figure S1*. Transplantation into sub-lethally irradiated secondary syngeneic recipients was performed using a 1-to-1 mixture of bone marrow cells (1 x 10^6 cells) and splenocytes (1 x 10^6 cells) from 3 independent primary recipient mice.

Results

Using RNA-sequencing and confirmed by Sanger sequencing, we identified a *STAT3 Y640F* mutation in the hematopoietic NK/T cell line YT1 (*Online Supplementary Figure S2A*). We then investigated the mutational status of STAT3 in a large cohort of patients with myeloid or lymphoid disorders. Based on previously reported *STAT3* mutations, we performed Sanger sequencing of 5 coding exons of *STAT3* (exons 3, 6, 17, 21, 22), including the exons encoding the Src homology 2 (SH2) domain.

Among B-cell neoplasms, *STAT3* mutations were detected in 2.5% of patients with diffuse large B-cell lymphoma (2 of 79) (Table 1 and *Online Supplementary Figure S2A*). No *STAT3* mutation was found in T-cell rich B-cell lymphoma (n=3), follicular lymphoma (n=60), mantle cell lymphoma (n=19), marginal zone lymphoma (n=12), or lymphocytic B lymphoma (n=9) (Table 1). We identified *STAT3* mutations in 4 of 258 patients (1.6%) with T-cell neoplasms: 2 patients with a cutaneous CD30$^+$ ALK-negative anaplastic large cell lymphoma (cALCL ALK- CD30$^+$), one with a peripheral T-cell lymphoma not otherwise specified and one with a gamma-delta T-cell lymphoma (Table 1 and *Online Supplementary Figure S2A*). A clinical summary of *STAT3* mutated patients is reported in *Online Supplementary Table S3*. For one of the cALCL ALK- CD30$^+$ patient, the blood cells in which no tumoral cells were detected did not present the mutation (*Online Supplementary Figure S2A*), further suggesting that the *STAT3* mutation was acquired in the malignant cells. A *STAT3 G618R* mutation was also identified in FEPD, an ALK- ALCL cell line (*Online Supplementary Figure S2A*). All the mutations identified in patient cells were heterozygous and were located in the SH2 domain (*Y640F, Y657ins, E616del (x2), D661ins, D661Y*). The position D661 was recurrently affected in accordance with the recent report

Table 1. STAT3 mutations in lymphoid and myeloid neoplasms.

Diagnosis	All patients	STAT3 mutated
B-cell neoplasms		
DLBCL	79	2 (2.5%)
TCRBL	3	0
FL	60	0
MCL	19	0
MZL	12	0
Lymphocytic	9	0
Unspecified B-cell lymphoma	1	0
T-cell neoplasms		
AITL	30	0
ALCL ALK$^+$	17	0
ALCL ALK-	6	0
ATLL HTLV1$^+$	2	0
TGLL	5	0
TPLL	25	0
PTCL, NOS	32	1 (3.1%)
Sezary syndrome	13	0
Mycosis Fungoides	10	0
cALCL ALK-	10	2 (20%)
Others mature T-cell lymphoma	13	1 (7,7%)
T-ALL/LBL	95	0
Myeloid malignancies		
PMF Jak2 unmutated	7	0
ET Jak2 unmutated	40	0
CMML	49	0
Total	537	6 (1.1%)

DLBCL: diffuse large B-cell lymphoma; TCRBL: T cell-rich B-cell lymphoma; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone lymphoma; AITL: angioimmunoblastic T-cell lymphoma; ALCL ALK$^+$: anaplastic large cell lymphoma ALK positive; ALCL ALK$^-$: anaplastic large cell lymphoma ALK negative; ATLL: adult T-cell lymphoma/leukemia (HTLV1$^+$); TGLL: T-cell large granular lymphocytic leukemia; TPLL: T-cell prolymphocytic leukemia; PTCL, NOS: peripheral T-cell lymphoma not otherwise specified; cALCL ALK$^-$ CD30$^+$: cutaneous anaplastic large cell lymphoma ALK negative CD30 positive; T-ALL/LBL: precursor T-cell acute lymphoblastic leukemia/lymphoma; PMF: primary myelofibrosis; ET: essential thrombocytosis; CMML: chronic myelomonocytic leukemia.

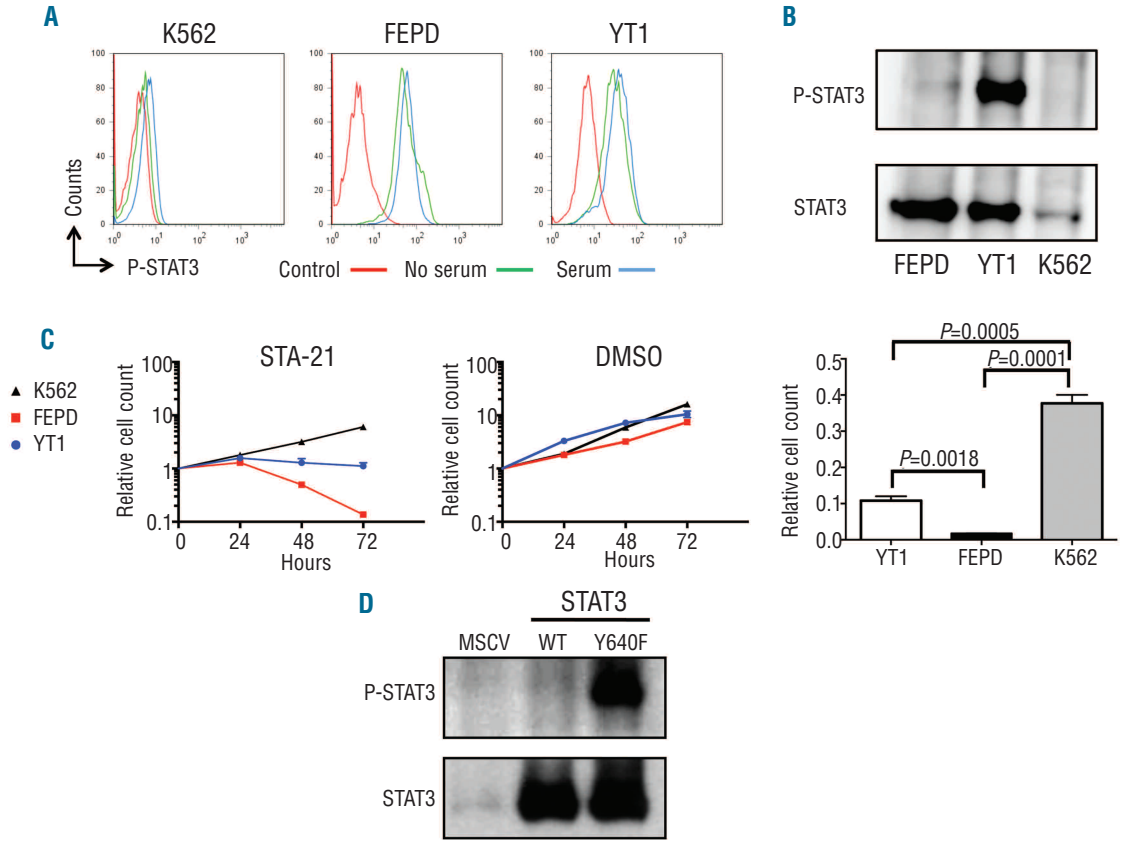

Figure 1. (A). **STAT3 phosphorylation analysis by flow cytometry of YT1 and FEPD (*STAT3* mutated cell lines) as compared to K562 (*STAT3* WT cell line). Control represents the unstained cells. Analysis was performed in normal conditions and after serum starvation.** (B). **STAT3 phosphorylation analysis by Western Blotting of YT1 and FEPD (*STAT3* mutated cell lines) as compared to K562 (*STAT3* WT cell line).** (C). **Proliferation assay of YT1, FEPD (*STAT3* mutated cell lines) and K562 (*STAT3* WT cell line) after treatment with the STAT3 inhibitor STA-21 (25 μM) (left panel) as compared to DMSO treatment (middle panel). The right panel shows the relative cell counts after 72-h of STA-21 treatment normalized to the number of DMSO-treated cells for each cell line.** (D). **STAT3 phosphorylation analysis by Western blotting of transduced murine Ba/F3 cells with empty (MSCV), wild-type (WT) STAT3 or STAT3 Y640F retroviruses.**

by Koskela *et al.*[4] and the missense mutation located at E616 had also been previously described in one patient with DLBCL.[6] No mutation was observed in myeloid malignancies, including wild-type *JAK2* primary myelofibrosis (PMF) (n=7), wild-type *JAK2* essential thrombocytemia (ET) (n=40) and chronic myelomonocytic leukemia (n=49). These results were consistent with a previous study that did not detect mutation of the JAK/STAT pathway (*JAK1, JAK3, TYK2, STAT5A,* and *STAT5B*) in wild-type *JAK2* ET and PMF patients.[7]

To investigate the functional consequences of *STAT3* mutations, we then analyzed STAT3 activation and phosphorylation in the YT1 and FEPD cell lines. Both lines showed constitutive STAT3 phosphorylation by flow cytometry (Figure 1A) and Western blotting (Figure 1B). Importantly, treatment of YT1 and FEPD with STA-21, a STAT3 small molecule inhibitor, resulted in a proliferation arrest of both STAT3 mutated cell lines, whereas the *STAT3* wild-type K562 cell line still proliferated (Figure 1C). Of note, STA-21 treatment is significantly more effective in FEPD in which the *STAT3* mutation is homozygous than in YT1 presenting a heterozygous *STAT3* mutation (Figure 1C and *Online Supplementary Figure S2A*). These results suggest that *STAT3* mutations induce a constitutive phosphorylation of STAT3 and participate in the proliferation of YT1 and FEPD cells.

Next, we transduced the IL3-dependent murine Ba/F3 cells with empty (control), wild-type (WT) *STAT3* or *STAT3* Y640F MSCV retroviruses. Western blotting confirmed that ectopic expression of *STAT3 Y640F* mutant induced constitutive phosphorylation of STAT3 compared to WT STAT3 and control cells (Figure 1D). Expression of STAT3 Y640F did not affect the cytokine dependency of the Ba/F3 cells, and no difference in proliferation was observed when BA/F3 cells were cultured in the presence of IL4 or FLT3 ligand (*Online Supplementary Figure S2B*). These results indicate that although *STAT3 Y640F* induced constitutive phosphorylation of STAT3, this mutant is not sufficient to confer cytokine-independent growth to Ba/F3 cells.

To investigate the role of *STAT3* mutations *in vivo*, we performed bone marrow transplantation (BMT) assays using bone marrow cells from 5-fluorouracil treated wild-type C57BL/6 mice transduced with *STAT3* Y640F, *STAT3* WT or empty retroviruses and injected to lethally irradiated syngenic recipients (*Online Supplementary Figure S1*). All the 10 STAT3 Y640F recipients developed symptoms of hematologic disease within one to three months and 9 were sacrificed between one and seven months after BMT for analysis. STAT3 Y640F mice presented MPN-like dis-

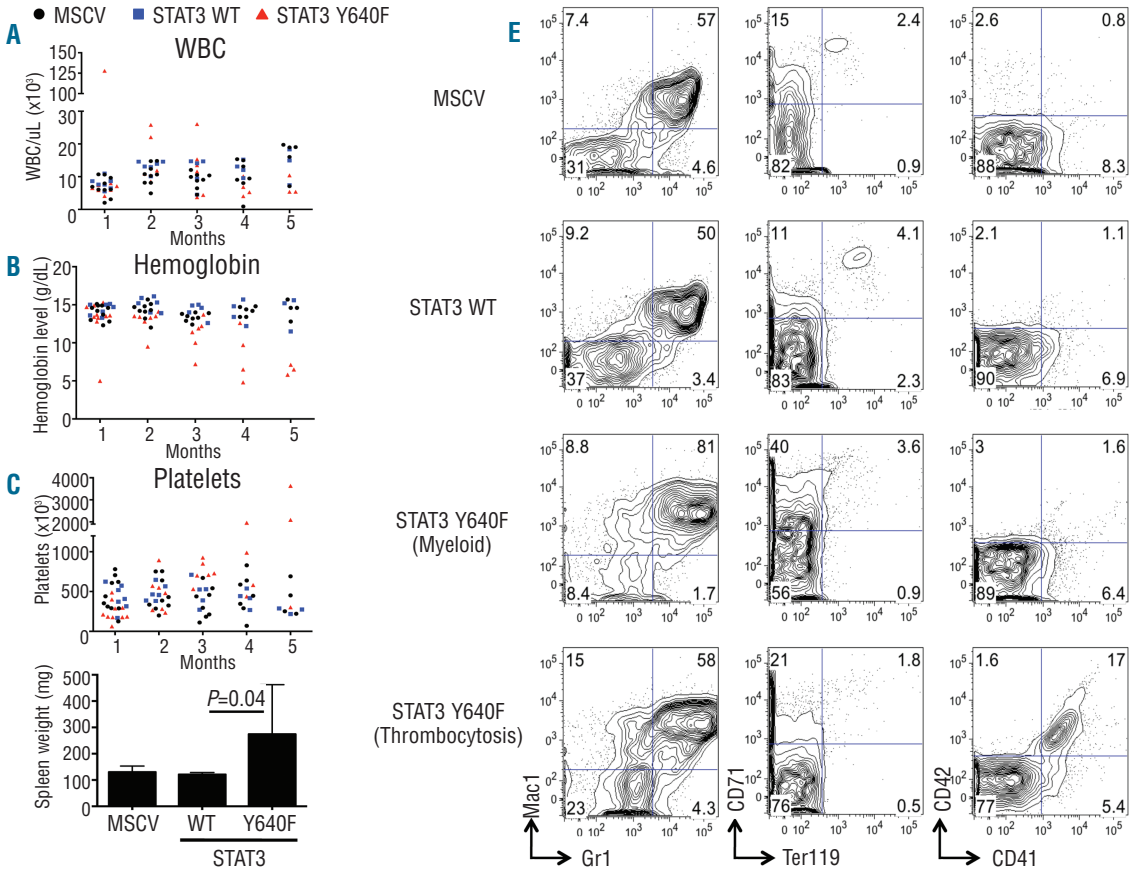

Figure 2. (A). White blood cells (WBC) count in peripheral blood samples obtained from MSCV, STAT3 WT and STAT3 Y640F mice. The x-axis values represent the number of months after bone marrow transplantation. (B). Hemoglobin level in peripheral blood samples obtained from MSCV, STAT3 WT and STAT3 Y640F mice. The x-axis values represent the number of months after bone marrow transplantation. (C). Platelet count in peripheral blood samples obtained from MSCV, STAT3 WT and STAT3 Y640F mice. The x-axis values represent the number of months after bone marrow transplantation. (D). Spleen weight of MSCV, STAT3 WT and STAT3 Y640F mice. (E). Representative flow cytometrical analysis of the mature myeloid cells (left), erythroid (center) and megakaryocytic (right) lineages in the bone marrow of STAT3 Y640F mice with myeloid and thrombocytosis diseases, as compared to MSCV and STAT3 WT mice. The percentages of GFP-positive cells are indicated.

eases with slight hyperleukocytosis (except for one mouse) (Figure 2A), a trend toward anemia (Figure 2B) and a significant splenomegaly (P=0.04, unpaired t-test with Welch's correction) (Figure 2D). Two distinct phenotypes were observed. Three mice developed a thrombocytosis disorder characterized by a progressive increase of the platelet counts (Figure 2C) associated with a severe anemia (Figure 2B). One animal, which initially presented thrombocytosis four months after BMT, showed platelet counts returning to normal with a concomitant decrease in the number of GFP positive cells (9-month follow up, *Online Supplementary Figure S2C*). Flow cytometry analysis of this subgroup indicated an alteration of the erythroid lineage maturation with a reduction in $CD71^{+}Ter119^{+}$ erythroblasts and a marked increase in $CD41^{+}CD42^{+}$ bone marrow megakaryocytes (Figure 2E). Seven mice presented a myeloid disease, with a transient hyperleukocytosis (except for one animal that succumbed early after BMT with a high hyperleukocytosis) (Figure 2A), a progressive anemia (Figure 2B), and trend toward thrombocytopenia (Figure 2C). Flow cytometry analysis indicated an abnormal $Mac^{+}Gr1^{+}cKit^{-}$ population infiltrating the bone marrow (Figure 2E), spleen and blood (*data not shown*), and defects in erythroid lineage maturation with accumulation of $CD71^{+}Ter119^{low}$ cells in the bone marrow (Figure 2E). No symptoms of disease or hematologic abnormalities were detected in empty MSCV or STAT3 WT recipients within this time lapse (Figure 2A-E). Also, no T-cell differentiation alteration was detected in either group. Importantly, the MPN-like diseases observed in primary STAT3 Y640F recipients, either thrombocytosis or myeloid diseases, could not be transplanted to secondary recipients with over six months of follow up (*Online Supplementary Figure S1C*). Together, these data indicate that *STAT3* Y640F induced an MPN-like disease in a bone marrow transplantation model.

Discussion

Here, we reported that *STAT3* mutations present a rare incidence in patients with lymphoid and myeloid diseases in general. We detected *STAT3* mutations in a rare subset of DLBCL patients (2.5%), which is consistent with recent analysis of DLBCL using high throughput genome sequencing[6,8] and in few patients with T-cell lymphoma. During the course of this study, *STAT3* mutations were reported as a frequent event in TGLL[4,9] and in chronic lym-

phoproliferative disorders of NK cells (CLPD-NK).[9] All observed *STAT3* mutations were located in exon 21, encoding the SH2 domain, and resulted in phosphorylation, dimerization and activation of the protein. Of note, no *STAT3* mutation was observed in our cases of TGLL, but this may result from the small number of analyzed patients. Our data also support the hypothesis that *STAT3* mutations participate in the tumorigenesis. Indeed, we demonstrate that the *STAT3 Y640F* mutation leads to constitutive phosphorylation of STAT3 and efficiently induces MPN with either myeloid or thrombocytosis features in a murine retroviral transduction-bone marrow transplantation model. Of note, these results are consistent with previous data showing the importance of STAT3 for granulopoiesis and megakaryopoiesis. Indeed, STAT3 has been described as an essential component of G-CSF-driven cell proliferation and granulopoiesis.[10] STAT3 is also important for both normal megakaryocyte development[11] and in some murine models of ET.[12] Of note, mice homozygous for a truncation mutation in gp130, deleting all STAT3 binding sites, show altered platelet production.[13] In addition, the expansion in the number of immature hematopoietic progenitor, thrombocytosis, and splenomegaly observed in a murine model of constitutive activation of gp130 can be rescued by genetic ablation of *STAT3*.[14] Although these data strongly suggest that STAT3 has a cell-autonomous contribution to normal and malignant megakaryocyte development, we cannot exclude the possibility that aberrant release of cytokines participates in the thrombocytosis observed in some mutant STAT3 recipients.

In this bone marrow transplantation setting, we observed the development of myeloid diseases without T- or NK-cell pathologies. This discrepancy has been observed in other conditions. For example, gain-of-function *JAK2* alleles detected in patients with Down's syndrome acute lymphoblastic leukemia induced myeloproliferative disorders using a similar bone marrow transplant setting.[15] Therefore, further studies are needed to clarify the role of *STAT3* mutations in the transformation of lymphoid lineages and will likely require expression of these *STAT3* mutant specifically in lymphoid lineages or their co-expression with co-operating mutations. Identification of the surface receptor interacting with STAT3 may also provide a better understanding of the constitutive activation of STAT3 observed in these human hematopoietic malignancies. Finally, these data may also be clinically relevant. Indeed, we observed that the proliferation of T-cell lymphoma cell lines presenting a *STAT3* mutation is inhibited by the STAT3 inhibitor STA-21. As *STAT3* mutations affect 40% of TGLL patients and 30% of CLPD-NK patients, it will be interesting to investigate further the efficacy of STAT3 inhibitors as a novel targeted therapy in these specific and rare pathologies.

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References

1. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3(7):e270.
2. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011; 118(7):1723-35.
3. Pilati C, Amessou M, Bihl MP, Balabaud C, Nhieu JT, Paradis V, et al. Somatic mutations activating STAT3 in human inflammatory hepatocellular adenomas. J Exp Med. 2011;208(7):1359-66.
4. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmaki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med. 2012;366(20):1905-13.
5. Steensma DP, McClure RF, Karp JE, Tefferi A, Lasho TL, Powell HL, et al. JAK2 V617F is a rare finding in de novo acute myeloid leukemia, but STAT3 activation is common and remains unexplained. Leukemia. 2006; 20(6):971-8.
6. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476(7360):298-303.
7. Scott LM, Campbell PJ, Baxter EJ, Todd T, Stephens P, Edkins S, et al. The V617F JAK2 mutation is uncommon in cancers and in myeloid malignancies other than the classic myeloproliferative disorders. Blood. 2005; 106(8):2920-1.
8. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci USA. 2012;109(10):3879-84.
9. Jerez A, Clemente MJ, Makishima H, Koskela H, Leblanc F, Peng Ng K, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood. 2012;120(15):3048-57.
10. Smithgall TE, Briggs SD, Schreiner S, Lerner EC, Cheng H, Wilson MB. Control of myeloid differentiation and survival by Stats. Oncogene. 2000;19(21):2612-8.
11. Kirito K, Osawa M, Morita H, Shimizu R, Yamamoto M, Oda A, et al. A functional role of Stat3 in in vivo megakaryopoiesis. Blood. 2002;99(9):3220-7.
12. Senyuk V, Rinaldi CR, Li D, Cattaneo F, Stojanovic A, Pane F, et al. Consistent up-regulation of Stat3 Independently of Jak2 mutations in a new murine model of essential thrombocythemia. Cancer Res. 2009; 69(1):262-71.
13. Jenkins BJ, Quilici C, Roberts AW, Grail D, Dunn AR, Ernst M. Hematopoietic abnormalities in mice deficient in gp130-mediated STAT signaling. Exp Hematol. 2002; 30(11):1248-56.
14. Jenkins BJ, Roberts AW, Najdovska M, Grail D, Ernst M. The threshold of gp130-dependent STAT3 signaling is critical for normal regulation of hematopoiesis. Blood. 2005;105(9):3512-20.
15. Malinge S, Ben-Abdelali R, Settegrana C, Radford-Weiss I, Debre M, Beldjord K, et al. Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. Blood. 2007;109(5):2202-4.