STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells

Christian Sillaber, Franck Gesbert, David A. Frank, Martin Sattler, and James D. Griffin

The transcription factor STAT5 is constitutively tyrosine phosphorylated and activated after transformation of hematopoietic cells by p210Bcr/Abl. A truncated form of STAT5B \(\Delta\)STAT5, aa, 1-683) that lacks tyrosine 699 and the transcriptional activation domain was introduced into Ba/F3p210 cells under the control of a tetracycline-inducible promoter. Treatment of these cells with doxycycline, a tetracycline analogue, induced expression of \(\Delta\)STAT5 and inhibited STAT5-dependent transcription. \(\Delta\)STAT5 coprecipitated with STAT5 and decreased Bcr/Abl-dependent tyrosine phosphorylation of endogenous STAT5. Induction of \(\Delta\)STAT5 inhibited growth of Ba/F3p210 cells (26%-52% of control levels at 4 days) but did not cause cell-cycle arrest. \(\Delta\)STAT5 reduced viability of Ba/F3p210 cells and increased sensitivity of the cells to the cytotoxic drugs hydroxyurea and cytarabine. These results indicate that high-level expression of \(\Delta\)STAT5, as achieved here by using a tetracycline-inducible promoter, inhibits STAT5 activity, reduces the growth rate of Ba/F3p210 cells by inhibiting viability, and results in increased sensitivity to chemotherapeutic drugs. It is therefore likely that STAT5 activation plays a role in the transformation of hematopoietic cell lines by p210Bcr/Abl. (Blood. 2000;95:2118-2125)

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Materials and methods

Cell lines and cell culture

Ba/F3 is an IL-3-dependent murine hematopoietic precursor cell line. Ba/F3 cells were maintained in RPMI 1640 medium (Mediatech Cellgro, Herndon, VA) supplemented with 10% (vol/vol) fetal calf serum and 10% (vol/vol) WEHI-3B-conditioned medium as a source of IL-3. Ba/F3 cells

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containing the reverse Tet transactivator pUHD172-1 (Ton.BaF.119) and Ton.B.210.1 cells,13 in which p210Bcr/Abl can be induced by the addition of doxycycline, were grown in the same medium. Transformed Ba/F3p210 cells are growth-factor independent and were maintained without WEHI-3B supernatant unless otherwise stated. All cell lines were grown in a humidified incubator at 37°C (5% carbon dioxide).

### Introduction of a truncated STAT5 mutant into Ba/F3p210 cells

A truncated form of the murine STAT5B (∆STAT5; aa, 1-683, in plasmid pBABE) was obtained from A. L. Mui18 (DNAX Research Institute, Palo Alto, CA). The construct was cloned into pTRE plasmid (Clontech, Palo Alto, CA) by using EcoRI and XhoI sites. The pTRE plasmid containing the truncated STAT5 construct was then cotransfected with a p210Bcr/Abl plasmid (pGD210p) into Ton.BaF.1 cells containing the reverse Tet transactivator.19 Cell lines were then selected for growth-factor independence in the absence of doxycycline. Factor-independent sublines were analyzed for doxycycline-inducible ∆STAT5 expression by Western blotting, as well as for expression of p210Bcr/Abl.

### STAT5 reporter gene assay

STAT5-dependent transcription was measured by using a reporter gene construct (GAS-luciferase) containing 4 tandem β-casein-like GAS elements from the β-globin locus control region cloned into the pGL2 luciferase vector (from A. D’Andrea, Boston, MA).21 This construct (20 µg) was transiently transfected into Ba/F3p210 cells. Twelve hours later, transfected cells were divided into 2 equal cultures and then maintained in either the presence or absence of doxycycline (1 µg/mL) for the next 24 hours. After being washed twice with phosphate-buffered saline (PBS) at 4°C, cells were resuspended in lysis buffer E397 (Promega, Madison, WI). The lysates (20 µL) were then incubated with 300 µL of luciferase assay buffer (25 mmol/L of glycylglycine [pH 7.8], 15 mmol/L of potassium phosphate [pH 7.8], 15 mmol/L of magnesium sulfate, 4 mmol/L of ethylene glycol tetraacetic acid, 2 mmol/L of adenosine triphosphate, and 1 mmol/L of dithiothreitol) and 100 µL of D-luciferin (0.3 mg/mL; Pharmingen, San Diego, CA). Luciferase activity was assessed with an automated luminometer (Lumat LB 9507; EG&G Berthold, Gaithersburg, MD). The plasmid CMVβ-GAL (20 µg; Invitrogen, San Diego, CA) was used as a reporter for transfection efficiency, and β-GAL activity was measured with a β-GAL assay kit (Invitrogen, Carlsbad, CA). When STAT5 reporter gene activity in 2 different cell lines was compared, luciferase activity was reported as a ratio of luciferase activity to βGAL activity.

### Preparation of cell lysates and Western blotting

Cells were washed twice in Dulbecco’s PBS at 4°C and resuspended in lysis buffer (1 mL/10^6 cells) consisting of 50 mmol/L of Tris (pH 8.0), 150 mmol/L of sodium chloride (NaCl), 1% (vol/vol) NP-40, 0.5% (wt/vol) deoxycholic acid, 0.1% (wt/vol) sodium dodecyl sulfate (SDS), 1% (vol/vol) sodium fluoride, 1 mmol/L of phenylmethylsulfonyl fluoride, 20 µg/mL of aprotinin, 40 µg/mL of leupeptin, and 1 mmol/L of sodium orthovanadate. Lysates were then incubated on ice for 30 minutes (resus- pended vigorously every 5 minutes), centrifuged for 15 minutes (12 000g), and washed 3 times with 1% (vol/vol) NP-40 at 4°C. Lysates and immunoprecipitates were then separated under reducing conditions by SDSD-polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane (Protran; Schleicher & Schuell, Keene, NH) in buffer containing 25 mmol/L of Tris, 192 mmol/L of glycine, and 20% (vol/vol) methanol at 4°C. The membrane was blocked for 1 hour in 5% (wt/vol) nonfat dry milk powder in Tris-buffered saline (10 mmol/L of Tris-hydrochloric acid [pH 8.0] and 150 mmol/L of NaCl). The membrane was incubated sequentially with the primary and the secondary horseradish peroxidase (HRP)-coupled Ab (1:5000 dilution in Tris-buffered saline; Amersham, Piscataway, NJ) for 1 hour. HRP activity was detected by using HRP substrates (Renaissance; NEN, Boston, MA) and X-Omat film (Kodak, Rochester, NY).

### Generation of stable Ba/F3p210 cells inducibly expressing ∆STAT5

The Ba/F3 cell line is a nonleukemic hematopoietic cell line that requires murine IL-3 for growth and viability. Transfection of Ba/F3 cells with p210Bcr/Abl makes these cells leukemic and growth-factor independent.22 To investigate the contribution of STAT5 activation to Bcr/Abl transformation, a series of cell lines was created in which a truncated, dominant negative, mutant form of STAT519 (∆STAT5, a C-terminal deletion that retains aa 1-683 of STAT5B) was expressed under the control of a doxycycline-inducible promoter. Ba/F3 cells stably expressing the reverse Tet transactivator pUHD172-1 (Ton.BF.119) were cotransfected with a ∆STAT5 construct (pTRE-∆STAT5) and p210Bcr/Abl (pGD210) and selected for growth-factor independence. These cells were designated Ba/F3p210∆STAT5 cells. As a control, TonBaF1 cells were transfected with pGD210 and the empty pTRE plasmid (designated Ba/F3p210Ctrl cells). Eight subclones of Ba/F3p210∆STAT5 cells were obtained by single-cell cloning. On induction with doxycycline (1 µg/mL), the truncated form of STAT5 (∆STAT5 [78 kd]) was induced in 3 of 8 clones tested. Inducible ∆STAT5 expression in these 3 subclones (C7.14, C7.28, and C7.30) is shown in Figure 1 (lower panel). Doxycycline did not induce any form of STAT5 protein in Ba/F3p210Ctrl cells. In the absence of doxycycline, ∆STAT5 expression in Ba/F3p210∆STAT5 cell clones was minimal but detectable, indicating a small degree of leakiness in this system. Each clone expressed endogenous (full-length) STAT5A and STAT5B (Figure 1, lower panel), p210Bcr/ Abl, and c-abl (upper panel). Growth-factor independence was due specifically to Bcr/Abl, since each of these clones died rapidly in the presence of the Bcr/Abl kinase inhibitor STI57123 (1 µmol/L). The expression levels of c-abl and Bcr/Abl in the 3
STAT5 binding sites in their promoters include β-casein, IRF1, CIS, and Bcl2. The STAT5 binding site of the β-casein gene has previously been used for STAT5 reporter gene constructs.

In the current study, we used a reporter gene construct (GAS-luciferase) in which a tandem repeat of 4 STAT binding motifs was cloned upstream of the luciferase gene in the pGL2 promoter vector. Using this reporter gene construct, we analyzed p210Bcr/Ab1-transformed cells for STAT5 reporter gene activity and compared the results with findings in nontransformed Ba/F3 cells. A β-GAL reporter gene construct was used as a transfection control. STAT5 reporter gene activity was considerably higher in Ba/F3p210 cells (Figure 3A), (ratio of STAT5 to β-GAL, 1533 ± 423) compared with untransformed Ba/F3 cells (ratio of STAT5 to β-GAL, 157 ± 30). Increased STAT5 reporter gene activity could directly be attributed to Bcr/Ab1, since incubation with STI571 (1 µmol/L for 12 hours) inhibited STAT5 activity (ratio of STAT5 to β-GAL, 312 ± 51) in Ba/F3p210 cells but had no effect in untransformed Ba/F3 cells (Figure 3A), (ratio of STAT5 to β-GAL, 157 ± 28).

The ability of Bcr/Ab1 to induce STAT5-dependent gene transcription was confirmed by using a cell line (TonB.210) in which p210Bcr/Ab1 can be induced by the addition of doxycycline. Doxycycline (1 µg/mL for 24 hours) significantly increased STAT5 reporter gene activity (Figure 3B) (ratio of STAT5 to β-GAL, 2089 ± 468 for the control and 10 216 ± 1805 for doxycycline-treated cells). Addition of doxycycline had no effect on untransformed Ba/F3 cells (Figure 3B) (ratio of STAT5 to β-GAL, 2476 ± 285 for the control and 1982 ± 276 for doxycycline-treated cells).

To determine whether induction of ΔSTAT5 in Bcr/Ab1-transformed cells would inhibit STAT5 reporter gene activity, the Ba/F3p210ΔSTAT5 clones were transfected with the β-casein reporter gene construct. Twelve hours after transfection, cells were

**Inhibition by ΔSTAT5 of Bcr/Ab1-dependent activation of a STAT5 reporter gene**

Phosphorylation of STAT5 leads to dimerization and translocation into the nucleus, where STAT5 dimers then activate transcription through binding to specific DNA sequences. The consensus STAT5 binding site is TTCNNNGAA. Genes known to have STAT5 binding sites in their promoters include β-casein, IRF1, CIS, and...
control; C7.28, 28.2%

D

ited STAT5 reporter gene activity. D

(99.5%

reporter gene activity was found in the Ba/F3p210Ctrl cells

42.0%

6

from 3 clones of Ba/F3p210

Figure 4. Inhibition of STAT5 reporter gene activity by doxycycline.25 Binding of CIS protein to the cytoplasmic domain of a cytokine receptor is associated with inhibition of STAT phosphorylation, thereby creating a negative feedback loop that limits
cytokine-induced STAT activation. The CIS gene, like the β-casein gene, has STAT5 binding sites upstream of its initiation site,24,26 and STAT5 activation leads to CIS expression.29 In the current study, CIS expression was constitutively expressed in Ba/F3p210 cells (Figure 5A), and induction of ΔSTAT5 with doxycycline for 24 hours reduced CIS protein expression (Figure 5A). Two forms of CIS, a 37-kd form and a 45-kd form, were detected by immunoblotting. The 45-kd form of CIS is likely to represent a ubiquitinated form of CIS.30 These results show that expression of ΔSTAT5 is associated with reduced transcription of a known split and then maintained in either the presence or absence of doxycycline (1 µg/mL) for 24 hours. ΔSTAT5 expression significantly reduced luciferase reporter gene activity in each of the 3 clones tested (Figure 4), [C7.14, 23.9% ± 9.5% of unstimulated control; C7.28, 28.2% ± 3.5% of unstimulated control; and C7.30, 42.0% ± 5.8% of unstimulated control]. No reduction of STAT5 reporter gene activity was found in the Ba/F3p210Ctrl cells (99.5% ± 18.6% of unstimulated control). Therefore, doxycycline-induced expression of ΔSTAT5 in Ba/F3p210ΔSTAT5 cells inhibited STAT5 reporter gene activity.

Inhibition of CIS protein expression but not of BclX protein expression in Ba/F3p210 cells by induction of ΔSTAT5

CIS is a protein involved in the negative regulation of JAK/STAT signaling.31 Binding of CIS protein to the cytoplasmic domain of a cytokine receptor is associated with inhibition of STAT phosphorylation, thereby creating a negative feedback loop that limits

Figure 3. Induction of STAT5 reporter gene activity with Bcr/Abl. (A) Ba/F3p210 cells and untransformed Ba/F3 cells in RPMI plus WEHI (10%) were transfected with the β-casein luciferase reporter gene (together with the p-β-GAL construct). Twelve hours after transfection, cells were split and maintained in either the presence or absence of Bcr/Abl kinase inhibitor STI571 (1 µmol/L). Cells were then harvested, and lysates were assayed for luciferase and β-GAL activities, expressed as a ratio of luciferase activity to β-GAL activity. Ba/F3p210 cells expressed significantly more STAT5 reporter gene activity than did Ba/F3 cells, and the increased STAT5 activity was due directly to Bcr/Abl, since it could be abrogated by Bcr/Abl kinase inhibitor STI571. (B) Untransformed Ba/F3 cells and T on B.210 cells were transfected with the β-casein luciferase reporter gene (together with the p-β-GAL construct). Twelve hours after transfection, cells were split, maintained in either the presence or absence of doxycycline (1 µg/mL), and assayed for luciferase and β-GAL activities. Induction of Bcr/Abl in T on B.210.1 cells by doxycycline increased STAT5 reporter gene activity. Doxycycline had no effect on STAT5 reporter gene activity in untransformed Ba/F3 cells.

Figure 4. Inhibition of STAT5 reporter gene activity by ΔSTAT5 expression. Cells from 3 clones of Ba/F3p210ΔSTAT5 cells were transfected with the β-casein luciferase construct. Twelve hours after transfection, cells were split and maintained in either the presence or absence of doxycycline (1 µg/mL) for 24 hours. Cells were then assayed for luciferase activity, and results are expressed as the percentage of activity in unstimulated controls. Induction of ΔSTAT5 in Ba/F3p210ΔSTAT5 cells by doxycycline significantly inhibited STAT5 reporter gene activity in all clones tested. No inhibition of STAT5 activity was observed when Ba/F3p210Ctrl cells were exposed to doxycycline.

Figure 5. Differential regulation of STAT5-induced genes, CIS and BclXL, in Bcr/Abl-transformed cells. (A) Inhibition of CIS protein expression by ΔSTAT5 expression. Lysates from Ba/F3p210ΔSTAT5 cells (after treatment with or without doxycycline [1 µg/mL] for 24 hours) were incubated with a CIS antibody and protein G beads. Immunoprecipitates were then subjected to SDS-PAGE (9%). Probing with anti-CIS antibody revealed constitutive expression of CIS protein (37 kd) in Bcr/Abl-transformed cells, and expression of ΔSTAT5 inhibited CIS protein expression. Addition of doxycycline had no effect on CIS protein expression in Ba/F3p210Ctrl cells. A larger, presumably ubiquitinated form of CIS protein (45 kd) was also expressed in Ba/F3p210 cells (upper right-hand panel). Induction of ΔSTAT5 with doxycycline (1 µg/mL for 3 days) had no effect on BclXL expression in Ba/F3p210ΔSTAT5 cells (upper right-hand panel). Induction of ΔSTAT5 in Ba/F3p210ΔSTAT5 cells is shown as a control (lower panel).
STAT5-inducible gene, which is consistent with the idea that ΔSTAT5 functions as a dominant negative mutant.

It was previously reported that the BclX gene, like the CIS gene, contains STAT5 binding sites,24 and induction of a constitutive active STAT5 mutant was found to enhance BclX levels.24 Bcr/Abl also induces BclX (Figure 5B, upper left panel), suggesting the possibility that BclX induction might be due to STAT5 activation by Bcr/Abl. However, when ΔSTAT5 was induced in Ba/F3p210ΔSTAT5 cells (using 1 µg/mL of doxycycline for 3 days), no effect on BclX protein expression was found (Figure 5B, upper right panel). These results suggest that, in Bcr/Abl-transformed cells, induction of CIS depends on STAT5 activity, whereas expression of BclX does not.

Effects of ΔSTAT5 expression on the growth and cell cycle of Ba/F3p210 cells

To determine the effects of ΔSTAT5 induction on the growth of Ba/F3p210 cells, Ba/F3p210ΔSTAT5 cells were maintained in either the absence or presence of doxycycline (1 µg/mL), and daily cell counts were obtained. Cells expressing ΔSTAT5 were found to grow more slowly than untreated cells. After 4 days of culture, Ba/F3p210ΔSTAT5 cells induced with doxycycline (1 µg/mL) grew at rates that were 26% to 52% of the growth rates of untreated cells (Figure 6). The degree of growth inhibition was similar in each of the 3 clones tested. On day 4, the values were as follows: C7.14, 43.2% ± 8.3% of control; C7.28, 26.2% ± 6.8% of control; and C7.30, 51.9% ± 11.8% of control. In contrast, doxycycline had no significant effect on growth of Ba/F3p210Ctrl cells; on day 4, the value was 87.1% ± 17.8% of control.

To determine whether ΔSTAT5 induced cell-cycle arrest, cell-cycle analysis was performed by using a standard PI staining protocol and flow cytometry. ΔSTAT5 expression did not result in G1 cell-cycle arrest or other alterations in the distribution of cells in other phases of the cell cycle (not shown).

Reduction of viability of Ba/F3p210 cells by ΔSTAT5 expression

When Ba/F3p210ΔSTAT5 cells were maintained in the presence of doxycycline (1 µg/mL) for 2 days, a small but significant increase in annexin V–positive (apoptotic) cells was observed in all clones tested (Figure 7). The addition of doxycycline to clone C7.14 increased annexin V–positive cells from 8.4% ± 1.1% to 20.8% ± 4.9%; from 8.7% ± 0.5% to 22.5% ± 3.3% in clone C7.28; and from 12.0% ± 1.3% to 20.5% ± 3.2% in clone C7.30 (Figure 7). In contrast, doxycycline (1 µg/mL) had no significant effect on viability of Ba/F3p210Ctrl cells (control, 8.4% ± 0.8% annexin V–positive cells; and doxycycline-treated cells, 9.0% ± 1.8% annexin V–positive cells). The reduced viability associated with ΔSTAT5 is likely to have accounted for some or all of the reduced growth rate of Ba/F3p210ΔSTAT5 cells.

Because many cytotoxic drugs inhibit growth of cancer cells by inducing apoptosis, we wondered whether the presence of ΔSTAT5 would alter the sensitivity of Bcr/Abl-transformed cells to chemotherapeutic drugs used to treat CML. Ba/F3p210ΔSTAT5 cells were incubated with cytarabine in either the presence or absence of doxycycline (1 µg/mL). Thirty-six hours later, cell viability was analyzed by using annexin V/PI staining. Incubation of uninduced Ba/F3p210ΔSTAT5 cells with cytarabine resulted in a dose-dependent increase in apoptotic cells, as expected (Figure 8A). Induction of ΔSTAT5 by doxycycline enhanced the sensitivity of these cells to cytarabine and produced a moderate but reproducible shift in the dose-response curve. Doxycycline had no effect on the sensitivity of Ba/F3p210Ctrl cells to cytarabine. However, Ba/F3p210Ctrl cells were less sensitive to cytarabine than were uninduced Ba/F3p210ΔSTAT5 cells. This may represent clonal variation, or it could reflect low-level expression of ΔSTAT5 due to leakiness of the inducible vector. Similar results were obtained when Ba/F3p210ΔSTAT5 cells were incubated with various concentrations of hydroxyurea for 5 hours. The percentage of apoptotic cells was higher after ΔSTAT5 induction than in cells not subjected to induction (Figure 8B). Viability in response to hydroxyurea did...
A tetracycline-inducible promoter was used to regulate expression of a truncated form of STAT5B in Ba/F3p210 cells. This mutant was truncated at aa 683 and lacked the transcriptional activation domain and the major tyrosine phosphorylation site of STAT5B at aa 699. ∆STAT5 functioned as a dominant negative inhibitor of wild-type STAT5 function, since it inhibited GAS-luciferase activity and also reduced expression of CIS, a STAT5-regulated gene. Induction of ∆STAT5 resulted in a slower growth rate but did not induce cell-cycle arrest. This finding suggested that ∆STAT5 might reduce viability, and when viability was analyzed by the sensitive technique of annexin V staining, expression of ∆STAT5 resulted in a reduction in viability sufficient to account for the slower growth rate. Moreover, induction of ∆STAT5 activity was associated with increased sensitivity to the apoptotic effects of hydroxyurea and cytarabine, 2 chemotherapeutic drugs commonly used to treat CML. A reduction in viability with ∆STAT5 expression in Bcr/Abl-transformed 32Dc13 cells was observed by Nieworowska-Skorska et al.31 However, in contrast to what we observed in Ba/F3p210 cells, ∆STAT5 induction in the 32Dc13p210 cells in their study led to inhibition of cell-cycle progression. The different results in the 2 studies could possibly be attributed to phenotypic differences between the cell lines used.

The Bcr/Abl oncogene exerts several biologic effects on hematopoietic cells that are partly dependent on the model system being studied. In cell-line models and in vivo studies in mice, Bcr/Abl is mitogenic, induces factor independence,32 inhibits apoptosis,32,33 alters integrin-mediated adhesion and motility.33 In primary CML cells, the major defects associated with Bcr/Abl are aberrant regulation of adhesion33 and possibly enhanced motility34 and viability.35 although not all investigators agree on the latter.35,36 Studies have suggested, however, that very immature primary myeloid cells from patients in the stable phase of CML are more likely to show enhanced viability and growth-factor independence.37 Despite the identification of many different signaling pathways activated by Bcr/Abl, it has been difficult to link any specific signaling event to a specific biologic effect. Thus, our results showing a link between constitutive activation of STAT5 and enhanced viability of Bcr/Abl-transformed cells are especially interesting.

Enhanced viability of myeloid lineage cells would be expected to result in an accumulation of cells with relatively normal function, in accordance with the known clinical phenotype of CML. In fact, available data suggest that Bcr/Abl activates several biochemical pathways that enhance viability. For example, Bcr/Abl is known to activate p21ras through binding of GRB2/SOS to tyrosine 177 of Bcr38 and probably also through SHP2, which is tyrosine phosphorylated by Bcr/Abl.38 Ras has been linked to viability signaling in a number of different cell types.39,40 Also, Bcr/Abl is known to activate PI3K, which initiates a pathway that enhances viability, possibly through activation of Akt and subsequent phosphorylation of Bad.3 Finally, Bcr/Abl induces expression of the antiapoptotic mitochondrial protein, Bcl-xL.41 Because the Bcl-xL promotor has STAT5 binding sites24 and we and others28 have shown that a constitutive active mutant of STAT5 can induce Bcl-xL, we wondered whether Bcr/Abl induction of STAT5 was required for increased Bcl-xL expression. Although we found that increased CIS expression was dependent on STAT5, expression of Bcl-xL was not.

These results suggest that the decreased viability induced by ∆STAT5 is not due to decreased levels of Bcl-xL and that the increased expression of Bcl-xL induced by Bcr/Abl is not mediated
only by STAT5. Overall, the finding of multiple signaling pathways to viability suggests that this is likely to be an important aspect of Bcr/Abl function and that inhibition of any single pathway is unlikely to eliminate the viability effect of this oncogene, as we showed here. This hypothesis can be tested more formally when mice with targeted disruption of genes involved in viability signaling pathways, including STAT5, are tested for sensitivity to Bcr/Abl transformation. Our data suggest that the defect will be small unless hematopoietic cells defective in multiple viability signaling pathways are used.

In normal hematopoiesis, the role of STAT5 remains unclear, since adult mice in which both alleles of STAT5A and STAT5B are inactivated by gene targeting have largely normal blood counts. However, STAT5 activation still seems to be required to support immature hematopoiesis, since the STAT5A and STAT5B double-knockout mice had a reduction in myeloid progenitor numbers in the bone marrow. Furthermore, a study has suggested that STAT5 is required for normal fetal erythropoiesis. Even if STAT5 turns out not to have a major role in postnatal hematopoiesis, constitutive activation of STAT5, as described here for CML cells, may confer an important phenotype. This idea is supported by a previous study showing that point mutations of the STAT5 molecule leading to a constitutively active form of STAT5 can support growth of Ba/F3 cells in the absence of murine IL-3. An analogous situation is that with IL-3 or GM-CSF: gene-targeting studies indicated that neither cytokine is required for normal hematopoiesis, but when the cytokines are expressed constitutively in transgenic mice, both cause a myeloproliferative disorder.

The mechanism whereby ΔSTAT5 functions as a dominant negative inhibitor of STAT5 function in Bcr/Abl-transformed cells is of interest. Mui and colleagues showed that ΔSTAT5 inhibits tyrosine phosphorylation of wild-type STAT5, and our current findings confirm those results. The mechanism of reduced tyrosine phosphorylation is unclear, but it is possible that ΔSTAT5 binds to the kinase (Bcr/Abl may or may not be the kinase in this case) and then fails to disengage because it lacks the major tyrosine phosphorylation site and is therefore not phosphorylated. ΔSTAT5 would thereby block binding and subsequent phosphorylation of wild-type STAT5. We also found here that a small amount of ΔSTAT5 can coprecipitate with STAT5, suggesting that heterodimers are formed either in vivo or in vitro. Because the transcriptional activation domain of ΔSTAT5 has been deleted, the resulting heterodimer would be expected to have reduced ability to transactivate STAT5-dependent genes. The formation of heterodimers presumably depends on the binding of the SH2 domain of ΔSTAT5 to phosphotyrosine SH2-binding sites on wild-type STAT5.

Overall, our data suggest a model in which STAT5 activation by Bcr/Abl helps maintain viability in hemopoietic cells, thereby contributing to myeloproliferative disease. It will be of interest to determine the exact mechanisms involved, particularly the role of Bcl2 and other viability genes that may be regulated by STAT5, and to use dominant negative mutants of STAT5 in the transformation of primary fetal and adult primary cells by activated Abl oncogenes. Such studies should complement studies in STAT5 knockout mice and provide a better understanding of the role of this pathway in CML. Finally, it will be important to determine the contribution of the STAT5 pathway relative to that of other viability signaling pathways, including p21ras and PI3K/Akt.

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