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STAT5 Activation by BCR-Abl Contributes to Transformation of K562 Leukemia Cells

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Signal transducers and activators of transcription (STATs) belong to a family of transcription factors that were originally identified as mediators of cytokine-induced gene expression. Recent evidence, however, has shown that certain members of the STAT family, including STAT3, are also involved in cellular transformation. Here we show that STAT5 also plays a role in cellular transformation by the BCR-Abl oncogene. In BCR-Abl transformed K562 cells, STAT5A and 5B are constitutively phosphorylated on tyrosine and are transcriptionally active. Moreover, expression of a dominant negative form of STAT5 shows that active STAT5 is necessary for the growth in soft agar of these cells. These results show that besides STAT3, STAT5 can also be involved in cellular transformation. © 1999 by The American Society of Hematology.

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MATERIALS AND METHODS

Cell culture. U937 and K562 cells were maintained in RPMI 1640 supplemented with 8% fetal calf serum (FCS) (HyClone, Greiner, Logan, UT). NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 8% FCS (Life Technologies, Breda, The Netherlands). K562 and NIH 3T3 cells were transfected with the expression plasmids pEGFP C1, pEGFP C1−STAT5A, pEGFP C1−STAT5B, pEGFP C1−STAT5A5B, and pEGFP C1−STAT5T5B using the calcium phosphate precipitation technique. The EGFP expression plasmid pEGFP C1 was used as a control. After transfection, the cells were selected for 100 μg/ml of G418 (Life Technologies). The transfected cells were grown in complete medium supplemented with 6 μg/ml of G418.

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performed as described previously.38 agar and scored as was described previously,40 except that RPMI 1640 bluescript SK
sion vectors for STA T3 or STA T5 were cotransfected instead of SK
DA TCA-3
8
bandshift assays:
tide was used in this study (only the upper strands are shown); for
Netherlands). Focus assays on NIH-3T3 cells were performed as
described previously. 22,38 Full-length cDNAs of the different STA Ts
Freiburg, Germany), pSG5-STA T3, pSG5-STA T3
b
carrier DNA. For some experiments, dominant negative expres-
sion vectors for STAT3 or STAT5 were cotransfected instead of
expression vectors for STAT3 or STAT5 were cotransfected instead of
bluescript SK— carrier DNA. For some experiments, dominant negative expres-
ble electroporation with 2 µg of reporter plasmid and 10 µg of bluescript SK— carrier DNA. For some experiments, dominant negative expres-
sion vectors for STAT3 or STAT5 were cotransfected instead of bluescript SK— carrier DNA. For some experiments, dominant negative expres-

RESULTS AND DISCUSSION

Previously, it was demonstrated that BCR/Abi activates STAT5, although activation of STAT1 was also reported in some cell types.7-14 To determine which STAT family members were activated in BCR/Abi-expressing K562 cells, we performed immunoprecipitation/Western blotting experiments using antisera specific for STAT1, 3, 5A, and 5B. Figure 1A shows that both STAT5A and 5B are phosphorylated on tyrosine residues in these cells. The second band observed in the STAT5B immunoprecipitation is likely to be caused by serine phosphorylation of STAT5B (data not shown). By contrast, activation of STAT1 or 3 could not be detected. These results were confirmed by gel-shift analysis. Figure 1B shows that STAT5A and 5B constitutively bind to the STAT binding site from the β-casein promoter and from the FcγRI promoter (not shown), while DNA binding of STAT1 and STAT3 could not be observed. These results clearly show that STAT5A and 5B are the major targets for BCR/Abi in K562 cells.

Although tyrosine phosphorylation and DNA binding of STAT5 was previously reported in BCR/Abi-expressing cells, transcriptional activation of STAT5-dependent promoters was not shown. We therefore transfected various STAT-dependent reporter constructs into K562 cells. Figure 2A shows that constructs containing STAT binding sites from the β-casein promoter (which can bind STAT1 and STAT5) and from the FcγRI promoter (GAS, which can bind STATs 1, 3, and 5) are more active than the TK-CAAT control reporter. By contrast, the IRE-CAT and SIE-CAT reporters, which cannot bind STAT5, are comparable to the TK-CAAT control reporter. These results suggest that STAT5 is indeed transcriptionally active in K562 cells. To further extend these results, we transfected K562 cells with the β-casein-CAT reporter together with either dominant-negative STAT5 (STAT5b750) or STAT3B (Fig 2B). While

**A**

K562

U937

STAT5A

STAT5B

STAT3

STAT1

IP

**B**

Antibody

1 2 3 4 5 6

Probe: β-casein

**Fig 1.** Constitutive activation of STAT5A and 5B in K562 cells. (A) K562 cells (3.10^6 per lane) and U937 cells (negative control) were lysed in RIPA buffer, after which the tyrosine phosphorylation state of different STAT molecules was assessed by immunoprecipitation and Western blotting. Probing the blot with an antiphosphotyrosine antibody clearly shows that only STAT5A and STAT5B are phosphorylated on tyrosine in K562 cells. (B) Nuclear extracts from K562 cells were analyzed in a gel shift assay using the STAT binding site from the β-casein promoter as a probe. Supershift analysis clearly shows that STAT5A and 5B constitutively bind to the β-casein site in K562 cells.
STAT3β did not significantly alter the activity of the β-casein-CAT reporter, STAT5b750 caused a strong repression of CAT activity, further suggesting that STAT5 is transcriptionally active in these cells.

In contrast with most nontransformed hematopoietic cells, one of the transformed properties of K562 cells is their ability to grow in semisolid media (soft agar). To determine whether activation of STAT5 is involved in this property of K562 cells, we used STAT5b750. K562 cells were transfected with STAT5b750, STAT3β, or the empty expression vector (LNCX), after which the transfected cells were selected in G418-containing media for 10 days. Thereafter, cells were plated in dishes containing soft agar. Figure 3 shows that K562 cells transfected with an empty expression vector (LNCX) grow efficiently in soft agar (Fig 3A and D). By contrast, K562 cells transfected with STAT5b750 form fewer and smaller colonies in soft agar (Fig 3B and D), suggesting that STAT5 is indeed involved in this aspect of cellular transformation by BCR/Ab1. As a control, cells transfected with STAT3β grew as efficiently in soft agar as vector controls, further showing that STAT3 is not involved in transformation by BCR/Ab1 (Fig 3C and D).

We next wanted to investigate whether STAT5 also contributes to cellular transformation by other oncogenes. Recently, we and others have shown that STAT3 is involved in cellular transformation by v-Src.18,22 Figure 4A shows that besides STAT3, STAT5A and 5B are also tyrosine-phosphorylated in v-Src-transformed NIH-3T3 cells, but not in the parental NIH-3T3 cells. However, in contrast to K562 cells, we failed to detect substantial STAT5 DNA binding activity in v-Src-transformed cells (data not shown). To investigate whether STAT5 is causally involved in v-Src–induced transformation, we performed focus assays in NIH-3T3 cells. Transfection of NIH-3T3 cells with v-Src efficiently induces focus formation in these cells (Fig 4B). As we have previously shown, this can be strongly repressed by cotransfecting STAT3β. Interestingly, cotransfection of STAT5b750 also represses v-Src–dependent focus formation in NIH-3T3 cells, albeit much less efficiently than STAT3β. The combination of STAT3β and STAT5b750 was somewhat more potent in repression focus formation than either plasmid alone. In addition, the repression observed with STAT5b750 could be overcome by cotransfection of wild-type STAT3, further suggesting that STAT5 indeed plays a role in transformation by v-Src. To rule out potential a-specific effects of STAT5b750 on STAT3-dependent signaling, we performed cotransfection of a STAT3-dependent reporter construct (SIE-CAT) together with v-Src, STAT3β, and STAT5b750. Figure 4C shows that STAT3β efficiently blocks v-Src–induced SIE-CAT activity. By contrast, STAT5b750 only slightly reduced v-Src–induced SIE-CAT activity, suggesting that STAT5b750 does not repress STAT3 function in these cells. These results show that although STAT3 is the major STAT involved in transformation by the v-Src oncogene, STAT5 is also likely to play a minor role.

Taken together, we have shown that besides STAT3, STAT5 is also involved in transformation mediated by at least two oncogenes. The mechanism by which active STAT5 contributes to cellular transformation remains to be determined, but is likely to involve constitutive activation of STAT5 target genes, which are somehow involved in the control of proliferation (eg, c-fos). In this respect, it is noteworthy that blocking STAT5 function in mouse BaF3 cells results in a significant decrease in IL-3–dependent proliferation,35 while a constitutively active form of STAT5 renders IL-3–dependent cells partially IL-3–independent.16 On the other hand, because STAT5 is involved in the regulation of the antipapoptotic Bcl2 homologue A1,42 enhanced survival and escape from apoptosis of cells containing active STAT5 might also contribute to cellular transformation. The availability of constitutively active STAT5 variants36,43 will undoubtedly give new insights into the mechanism by which STAT5 contributes to cellular transformation.
Fig 4. STAT3 is also involved in transformation by the v-src oncogene. (A) The tyrosine phosphorylation status of STAT3 and STAT5 was analyzed in NIH 3T3 cells and two derivatives that were transformed by the v-src oncogene. STAT3 and STAT5 are both constitutively phosphorylated on tyrosine in v-src-transformed, but not parental NIH-3T3 cells. (B) NIH 3T3 cells were transfected with v-src expression plasmid and increasing concentrations of DN-STAT3 and DN-STAT5 expression vectors or wild-type STAT5 as a control. Two weeks after transfection, foci were scored and represented as percent compared with v-src alone. Both DN STAT3 and DN STAT5 partially block transformation of NIH 3T3 by v-src, although DN STAT3 is much more potent. Wild-type STAT5 could overcome the effect of STAT5/750, but not STAT3/750. Only STAT3/750 is able to block v-src-induced SIE-CAT activity.

REFERENCES
12. Frank DA, Varticovski L: BCR/abl leads to the constitutive activation of Stat proteins, and shares an epitope with tyrosine phosphorylated Stats. Leukemia 10:1724, 1996


