JunB inhibits proliferation and transformation in B-lymphoid cells


The activator protein 1 (AP-1) member JunB has recently been implicated in leukemogenesis. Here we surveyed human lymphoma samples for expression of JunB and other AP-1 members (c-Jun, c-Fos, Fra1, JunD). JunB was strongly expressed in T-cell lymphomas, but non-Hodgkin B-cell lymphomas do not or only weakly express JunB. We therefore asked whether JunB acted as a negative regulator of B-cell development, proliferation, and transformation. We used transgenic mice that expressed JunB under the control of the ubiquitin C promoter; these displayed increased JunB levels in both B- and T-lymphoid cells. JunB transgenic cells of B-lymphoid, but not of T-lymphoid, origin responded poorly to mitogenic stimuli. Furthermore, JunB transgenic cells were found to be less susceptible to the transforming potential of the Abelson oncogene in vitro. In addition, overexpression of JunB partially protected transgenic mice against the oncogenic challenge in vivo. However, transformed B cells eventually escaped from the inhibitory effect of JunB: the proliferative response was similar in explanted tumor-derived cells from transgenic animals and those from wild-type controls. Our results identify JunB as a novel regulator of B-cell proliferation and transformation.

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tumor growth by cooperating with c-Jun in the development of murine fibrosarcoma. Finally, JunB is overexpressed in human Hodgkin lymphoma but it is apparently transcriptionally inactive. Thus, given the dual function of JunB and its cell-type–specific action, it is difficult to predict the role of JunB in transformation of lymphoid cells. In this study we have investigated the expression pattern of JunB in non-Hodgkin lymphomas and addressed the functional role of JunB within the B-lymphoid compartment.

Materials and methods

Patient samples and protein analysis

Samples of lymph nodes of patients with lymphoid malignancies were obtained from the Institute of Pathology, University Hospital Graz (Graz, Austria). The lymphomas were classified according to the Revised European-American Classification of Lymphoid Neoplasms (REAL) classification.

Cells were lysed in a buffer containing protease and phosphatase inhibitors (50 nM HEPES [N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid], pH 7.5; 0.1% Tween-20; 150 mM NaCl; 1 mM EDTA [ethylenediaminetetraacetic acid]; 20 mM β-glycerophosphate; 0.1 mM sodium vanadate; 1 mM sodium fluoride; 10 μg/mL each aprotinin and leupeptin; and 1 mM PMSF [phenylmethylsulfonyl fluoride]). Protein concentrations were determined using a bicinchoninic acid (BCA) kit as recommended by the manufacturer (Pierce, Rockford, IL). One hundred micrograms total protein/sample was electrophoretically resolved on polyacrylamide gels containing sodium dodecyl sulfate (SDS) and transferred onto Immobilon (Millipore, MA) membranes. Membranes were probed with the antibodies indicated in the figure legend (Figures 1-2, 4, 6-7). The antisera directed against abl, p16, p21, and p27 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), the antibody against JunB was a generous gift from M. Yaniv (Pasteur Institute, Paris, France), and the c-Jun and Bclx antibodies were obtained from Transduction Laboratories (Lexington, KY). Sites of antibody binding were detected using protein A–conjugated horseradish peroxidase (EY Laboratories, San Mateo, CA) with chemiluminescent detection (enhanced chemiluminescence [ECL] detection kit; Amersham, Arlington Heights, IL).

Histology and immunohistochemistry

Paraffin- and acrylate-embedded specimens were obtained from the Institute of Pathology, University Hospital Graz. Tissue array technology was employed to compare samples using antibodies against Ki-67, CD20, CD23, CD30, CD4 (Dako, Glostrup, Denmark), JunB, c-Jun, c-Fos, Fra-1, JunD (Santa Cruz Biotechnology), and the alkaline phosphatase anti–alkaline phosphatase technique. Samples were rated positive for the individual AP-1 members, when the staining intensity of the tumor cells was at least 20% of the positive control (human placental tissue). The density of JunB in non-Hodgkin lymphomas and addressed the functional role of JunB within the B-lymphoid compartment.

Analysis of pro-B cells

JunB transgenic and wild-type bone marrow was prepared (n = 3 of each genotype). One aliquot was subjected to FACS analysis immediately after preparation. The remaining cells were transferred to RPMI medium as described above and cocultured with IL-7–producing NIH3T3 cells as feeder layers. Seven days thereafter the cell culture consisted of 80% to 90% CD19+ cells as analyzed by FACS and was subjected to a [3H]thymidine incorporation assay. FACS

Single-cell suspensions of cells were preincubated with αCD16/CD32 antibodies (Pharmingen, Hamburg, Germany) to prevent nonspecific Fc receptor–mediated binding. Thereafter, aliquots of 5 × 10^7 cells were stained with monoclonal antibodies conjugated with fluorescent markers and analyzed by FACS (Becton Dickinson, San Jose, CA). The antibodies used for lineage determination included the B-cell lineage markers B220, CD19, and CD43; the T-cell markers CD4, CD8, and Thy1.2; the myeloid markers Gr1 and Mac1; and the erythroid-lineage marker Ter119 (all from Pharmingen).

Infection of fetal livers, in vitro transformation assays, and establishment of cell lines

For the preparation of fetal liver cells, heterozygous animals were set up for breeding and vaginal plugs were checked daily. Fifteen days after conception the pregnant animals were killed and the fetal livers prepared. The embryo tails were used for genotyping by polymerase chain reaction (PCR). Single-cell suspensions from fetal livers were infected for 30 minutes with viral supernatant derived from A010 cells enriched with 5 ng/mL IL-7, 7 μg/mL polybrene, and β-mercaptoethanol. The virus-infected as well as the mock-infected cells were then maintained for 15 to 20 hours on IL-7–producing feeder layers (T220-29 cells). Thereafter, cells were washed several times and plated in cytokine-free methylcellulose at a density of 2 × 10^5 cells/mL with Colorado-2000 medium. Ninety minutes thereafter the cells were exposed to 0.006 J/cm^2 in RPMI medium. Ninety minutes thereafter the cells were harvested and lysed for Western blot analysis.

B- and T-cell proliferation, [3H]thymidine incorporation

Splenic B and T cells were sorted for the expression of CD19 and CD3, respectively, by magnetic-activated cell separation (MACS) according to the manufacturer’s instruction (Miltenyi Biotech, Bergisch Gladbach, Germany). The purity of the cells after MACS was controlled by fluorescence-activated cell sorter (FACS) and was between 85% and 95% in the individual experiments. For thymidine incorporation assays, the cells were then plated at a density of 2 × 10^5 cells in 96 round-bottom wells and stimulated with the cytokines as indicated. The concentrations used were 100 ng/mL interleukin 4 (IL-4), 20 μg/mL α-immunoglobulin M (α-IgM), and 1 μg/mL α-CD40. [3H]Thymidine was added 48 hours after stimulation for another 12 hours. To test the proliferation of cytokine-independent tumor cells, 2 × 10^5 cells were plated in round-bottom 96-well plates; 18 hours thereafter [3H]thymidine was added and incubated for another 12 hours.

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Injection of tumor cells into nude mice

Ten days after infection, 1 × 10^6 cells were resuspended into 300 μL of PBS and injected subcutaneously into nude (nu/nu) mice. At the time point of injection the cells had been in growth factor-free medium for at least a week and consisted of CD19^+ /CD43^+ pro-B cells. Mice were checked daily for the development of tumors. Tumors bigger than 2 cm in diameter were excised for further analysis.32

Infection of neonatal mice with the Abelson oncogene (Ab-MuLV)

Newborn mice were injected retroperitoneally with 50 μL of replication-incompetent ecotropic retrovirus encoding for v-abl. The mice were then checked daily for onset of diseases. Sick mice were killed and analyzed carefully for signs of disease.30

Results

Low expression of JunB in human B-lymphoid malignancies

We investigated the expression of JunB, c-Jun, c-Fos, Fra-1, and JunD in human non-Hodgkin lymphomas by tissue-array technology using a collection sample covering the majority of non-Hodgkin B-lymphoid malignancies. The tissue samples comprised lymph node biopsies of patients suffering from follicular lymphomas (FLs), diffuse large-cell lymphomas (DLCls), B-cell chronic lymphoid leukemia (B-CLL), and the highly malignant large-cell anaplastic T-cell lymphomas (ALCLs). Normal resting lymphoid cells that surrounded the tumor cells were taken as internal controls and stained negative for all 4 of the AP-1 factors analyzed. We detected positive staining for c-Jun, c-Fos, Fra-1, and JunB in the majority of the cases. ALCL was strongly positive in more than 80%. Interestingly, JunB showed a characteristic expression pattern: JunB staining was absent or low in B-cell malignancies (0% in B-CLL, between 10% and 15% for DLBL and FL), whereas the T-cell lymphomas, ALCLs, expressed JunB strongly in 15 of 17 cases (Figure 1A-B). To confirm this observation using a different technique, we complemented our study by immunoblot analysis directed against JunB. Samples of tumor cells derived from patients suffering from FL, DLBL, B-CLL, and ALCL were analyzed by Western blot analysis of lymph nodes from patients suffering from different B-cell malignancies (Figure 1C). To confirm this observation using a different technique, we complemented our study by immunoblot analysis directed against JunB. Samples of tumor cells derived from patients suffering from FL, DLBL, B-CLL, and ALCL were analyzed by Western blot analysis of lymph nodes from patients suffering from different B-cell malignancies (Figure 1C).

Overexpression of JunB blocks proliferation in murine B- but not T-lymphoid cells

In order to investigate the mechanism accounting for the differences in the expression of JunB in B- and T-cell malignancies, we used a transgenic mouse model that allows us to study the impact of JunB in both lymphoid compartments in vivo. As can be seen in Figure 2A, Ubi-junB transgenic animals showed an increased expression of JunB in B- and T-lymphoid cells. The numbers of B-lymphoid cells in the peripheral blood of junB transgenic animals were found to be reduced to 60% when compared with age-matched wild-type littermate controls, whereas the numbers of peripheral T-lymphoid cells were in the same range (data not shown). Stimulation of T cells with increasing concentrations of the lectin concanavalin A resulted in comparable mitogenic responses for wild-type and transgenic cells (Figure 2B). In contrast, the proliferative response in peripheral B cells (ie, CD19^+ cells) was severely impaired. This effect was observed regardless of the proliferative stimuli for it was observed in the presence of increasing concentrations of lipopolysaccharide (LPS) and of the B-cell–specific stimuli IgM, anti-CD40 and IL-4 (Figure 2C-D).

We next addressed the proliferative capacity of pro-B cells isolated from bone marrow of JunB transgenic animals. It is noteworthy that the numbers of B-cell progenitors were slightly elevated in bone marrow preparations from JunB transgenic mice. This could reflect a positive growth-promoting role of JunB in early lymphoid progenitors (Figure 3A). However, stimulation with the pro-B-cell–specific interleukin-7 (IL-7) revealed a reduced proliferative capacity compared with wild-type controls (Figure 3B). Based on these 2 sets of observations we conclude that JunB exerts a growth-suppressing activity on cells starting from the pro-B-cell stage.

JunB overexpression is accompanied by an increased expression of the cyclin-dependent kinase inhibitor protein p16 in B cells

In fibroblasts maintained in culture, overexpression of JunB resulted in up-regulation of the cell cycle kinase inhibitor p16, which was proposed to mediate the growth-inhibitory effect of JunB.29 However, it is not known whether this effect is also seen in vivo and if it occurs in all cell types. Hence, we selected 4 tissues that reportedly expressed p16 in wild-type adult mice to screen for p16 mRNA levels by reverse transcriptase–PCR (RT-PCR; Figure 4A). Interestingly, transcripts encoding p16 were elevated in
Figure 2. JunB overexpression inhibits proliferation of mature peripheral B lymphocytes. (A) Spleens from Ubi-junB transgenic animals (JunB tg) and their littermate wild-type controls (wt) were used for MACS purification of T and B cells. Whole-spleen extracts (S), CD3–purified T lymphocytes (T), and CD19–purified B lymphocytes were subsequently subjected to Western blotting using an antibody directed against JunB. Extracts of wild-type and Ubi-junB transgenic fibroblasts were used as controls. (B) CD3+ T lymphocytes were MACS purified from spleens of Ubi-junB transgenic animals (n = 3; □) and their littermate wild-type controls (n = 3; □). Cells (2 × 10^6/well) were subsequently subjected to a [H]thymidine proliferation assay using increasing concentrations of concanavalin A. (C–D) CD19+ B-lymphoid cells were MACS sorted from spleen of wild-type (n = 3; □) and Ubi-junB transgenic (n = 3; □) mice. Cells (2 × 10^5/well) were stimulated with the factors indicated for 48 hours and subjected to a [H] thymidine incorporation assay. Co indicates control, cells that were plated in medium without the addition of growth factors. Data represent means ± SDs from 6 individual wells. One representative experiment is shown (n = 3).

Figure 3. The growth inhibitory effect of JunB on B lymphocytes extends to CD19+CD43+ B-cell precursors. (A–B) Bone marrow cells of wild-type (n = 3) and Ubi-junB transgenic (n = 3) mice were analyzed by FACS to evaluate the numbers of B-cell precursors. Numbers in the right corners show the amount of B-cell precursors in the bone marrow. One representative experiment is shown (n = 6) (A). The remaining cells were cocultivated on IL-7–producer cells for 7 days to enrich for B-cell precursors. After 7 days, the bulk cultures, which now consisted of 80% CD19+CD43+ cells, were subjected to [H]thymidine incorporation assay after stimulation with 10 ng/mL IL-7 (B). Data represent means ± SDs from 6 individual wells. One representative experiment is shown (n = 2).

Figure 4. JunB overexpression in B lymphocytes is accompanied by an increased expression of the cyclin-dependent kinase inhibitor protein p16. (A) Semiquantitative RT-PCR analysis of p16 mRNA levels in lung and spleen from Ubi-junB transgenic animals and their wild-type littermate controls. (B) B- and T-lymphoid cells were MACS sorted from spleen of wild-type and Ubi-junB transgenic mice and subjected to Western blot analysis (upper panel) for JunB, p16, and β-actin (loading control). One representative FACS analysis of the purified B- and T-lymphoid cells after MACS purification is depicted (lower panel). (C) B- and T-lymphoid cells were MACS sorted from spleen of wild-type and Ubi-junB transgenic mice, stimulated with growth stimuli as indicated, and subjected to Western blot analysis for JunB, p16, and β-actin (loading control).

JUNB transgenic mice are partially protected from v-abl–inflicted leukemia/lymphomas

An additional possibility to test for tumor formation by the Abelson oncogene is the direct injection of a replication-incompetent retrovirus into newborn mice. In vivo, this single exposure to the v-abl oncogene provokes the development of a B-cell lymphoma leukemia in early adulthood. We injected 11 JunB transgenic and 12 wild-type mice with v-abl oncogene to investigate whether the overexpression of JunB protects the mice from the oncogenic challenge. Most interestingly, while all of the wild-type mice died within 3 months, 3 of the transgenic animals survived the oncogenic challenge for more than 9 months (Figure 5B). In addition, the diseased transgenic animals had an increased mean survival time compared with their wild-type littermates (12.1 ± 0.8 versus 8.4 ± 1.7 weeks; P = .04).
the average numbers of mice were infected with Abelson retrovirus and subsequently cloned in cytokine-free vitro and tumor formation in vivo. Liver cell suspensions. Fetal livers from wild-type (n = 6) and JunB transgenic animals (n = 5) were affected to a lesser degree such that the regular organ parenchyma of the spleen and the portal fields in wild-type animals were heavily infiltrated by leukemic cells. In the wild-type liver, leukemic cells were spilling out past the limiting plates of the portal tracts. In contrast, in JunB transgenic animals, spleen and liver were affected to a lesser degree such that the regular organ architecture was maintained (Figure 5D). We therefore conclude that overexpression of JunB mitigated the severity of v-abl–induced disease. This resulted in a prolonged median survival time of JunB transgenic animals.

Abelson-transformed pro-B cells have escaped the growth inhibition by JunB

In order to understand why protection by JunB was incomplete, we explanted tumors and propagated the cells in vitro. It was evident that the tumor cells obtained from JunB transgenic animals had overcome the inhibitory effect of the transgene for we failed to detect any differences between the wild-type and JunB transgenic tumor cells in response to mitogenic stimulus (Figure 6A). Western blot showed a considerable variation between cell lines derived from JunB transgenic tumors. Regardless of this variability, the levels were consistently higher than in tumor cells from wild-type animals (Figure 6B, top). The discrepancy between high levels of JunB and normal proliferative capacity suggested that during transformation and expansion in vivo the tumor cells had overcome the inhibitory effect of the JunB transgene. In order to verify this conjecture, we determined the levels of p16, an established cell cycle inhibitors D2, E, and A and the cell cycle inhibitor protein p21 did not show any major change (data not shown).

v-abl–transformed B cells escape growth inhibition by JunB

We therefore speculated that the transformation process might render the cells insensitive to the growth-suppressing effect of JunB by inactivating the JunB-dependent pathway that is involved in cell cycle control. To prove this hypothesis, 3 clonal Abelson (v-abl)–transformed pro-B cell lines were infected with JunB-expressing retrovirus resulting in a 5– to 8-fold overexpression of JunB (Figure 7C). As predicted, JunB overexpression affected neither proliferation nor cloning efficiency in growth factor–free methylcellulose of the transformed pro-B cell lines (Figure 7A-B).

A strong induction of JunB can be achieved by UV irradiation. Eight wild-type cell lines expressing various levels of p16 were chosen and UV irradiated (Figure 7D). Irrespective of the expression levels of p16, no further increase was observed by the UV-induced JunB induction. In addition, we injected v-abl–transformed JunB transgenic and wild-type cell lines into nude mice and analyzed tumor formation after 2 weeks. Tumor onset was observed in 72% of the mice that had received JunB transgenic tumor cell lines in 67% of the nude mice that had obtained v-abl–transformed wild-type cells. Also, the tumor weight was comparable between both groups (Figure 7D). These experiments prove that v-abl–transformed cells had escaped growth inhibition by JunB and have lost the ability to react with elevated p16 levels to JunB expression.

Discussion

Hodgkin lymphoma cells express JunB (and c-Jun) to high levels. In contrast, loss of JunB leads to murine chronic myeloid leukemia. Thus, JunB may have Janus-like properties in hematologic cells. Here we have addressed the role of JunB in the development of T- and B-cell lymphoma. Our observations show that (1) non-Hodgkin lymphomas of B-lymphoid origin do not or only weakly express JunB, whereas a strong expression is found in lymphomas that originate from the T-lymphoid lineage; (2) JunB selectively blocks B-lymphoid, but not T-lymphoid, cell proliferation ex vivo; and (3) overexpression of JunB blunts the transforming ability of the B-lymphoid–specific form of the Abelson oncogene in vivo and in vitro.

It has long been known that AP-1 members, and JunB in particular, exert their actions in a cell-type–specific manner because they impinge on a large set of target genes that are coregulated by other transcription factors (eg, the glucocorticoid receptor). An additional level of complexity is brought about by the cell-type–specific expression of AP-1 members that results in a
The diverse set of dimeric combination partners. Our findings are consistent with this concept of cell-type-specific action, for JunB only exerted antiproliferative actions in B cells. In T cells, JunB is necessary for the induction of Th2-dependent cytokines, but the mitogenic response of T cells, in contrast, proved resistant to elevated JunB levels. Whereas a tight correlation of JunB and p16 expression levels is observed in B-lymphoid cells, T cells react with an up-regulation of JunB but a decrease of p16 protein to mitogenic stimulation.

Immature common lymphoid precursors may also be resistant to growth inhibition by JunB. In fact we suspect that high levels of JunB may have a growth-promoting effect in early lymphoid progenitors because the numbers of (CD19+/CD43+) B-cell precursors are elevated in the bone marrow of junB transgenic mice. In contrast, we found that the levels of (CD19+/CD43-) B cells were reduced by 40% in peripheral blood. Taken together, these observations are indicative of a partial block starting at the level of the pro-B-cell stage. Finally, it is worth mentioning that the variation in AP-1 members was also readily evident in human lymphoid malignancies. Tissue array staining showed that cells from B-CLL samples were negative for JunB but highly positive for Frla (28 of 28 samples). By comparison, anaplastic large-cell lymphoma (ALCL), a T-cell-derived malignancy, was characterized by the highest levels of JunB. Thus, 15 of 17 samples were strongly positive by tissue-array staining. These were also highly positive for c-Jun. Our data are consistent with a recent report that shows high AP-1 activity in 3 cell lines derived from ALCL.

Two additional findings support the argument that JunB is an inhibitor of B-lymphoid transformation. (1) Ex vivo transformation by a retrovirus-encoding v-abl is blunted if B cells overexpress JunB. We stress that this experimental strategy relies on the use of primary hematopoietic precursors. These fetal cells are unlikely to have already acquired any mutations that predispose them to transformation. (2) Accordingly, the difference between junB transgenic and wild-type B cells can be recapitulated in vivo. Overexpression of JunB mitigates the course of the disease. Three of 11 transgenic animals survived the oncogenic challenge for more than a year. In the remaining 8 Ubi-junB transgenic animals JunB overexpression delayed the development of leukemia/lymphoma resulting in an increased survival time. In contrast, all 12 wild-type littermate controls died within 12 weeks. Overexpression of JunB did not, however, completely prevent the development of leukemia/lymphoma in Ubi-junB transgenic animals. This is due to the fact that transformed cells can eventually overcome growth inhibition by JunB. This conclusion is supported by the following experimental findings. (1) When established JunB transgenic and wild-type tumor cell lines were injected into nude mice, we did not observe any difference in tumor onset, latency, or tumor weight. (2) Moreover, no differences in proliferation were observed in primary tumor cells explanted from the diseased Ubi-junB and diseased wild-type mice. This strongly indicates that the transgenic tumor cells had acquired the ability to circumvent growth inhibition by JunB. (3) Most conclusively, forced expression of JunB in established tumor cell clones did not affect cloning efficiency or cell proliferation. This finding unequivocally demonstrates that the transformed cells had overcome the JunB-dependent growth control. In murine B-lymphoid cells, the escape from growth inhibition by JunB is achieved by uncoupling of JunB elevation from p16 up-regulation. Even strong induction of JunB by UV light did not alter p16 levels, irrespective of its expression levels.

Further experiments need to determine whether this effect is specific for v-abl or whether elevated JunB levels are capable of interfering with transformation induced by other oncoproteins. According to the high expression levels of JunB in ALCL and the lack of p16 induction by mitogenic stimulation in T cells, we expect the tumor-suppressing action of JunB to be limited to the B-lymphoid lineage.

To the best of our knowledge, there is no comparable systematic survey of AP-1 members in a large set of non-Hodgkin lymphoma by tissue array. However, JunB expression was assessed in a limited number of human cutaneous lymphomas by DNA microarray. JunB expression was lost in 3 of 4 primary cutaneous B-cell lymphomas, whereas JunB was amplified in primary cutaneous T-cell lymphomas. Similarly, the level of JunB has been determined in tissue samples from patients with Hodgkin disease. The vast majority of Hodgkin lymphomas are thought to originate from B cells, although the pathogenesis has remained elusive. It has been noted that Hodgkin lymphomas express c-Jun and JunB at high levels. Regardless of the different status of Hodgkin and non-Hodgkin lymphoma, it is worth pointing out that these cells also escape from growth inhibition by JunB. In spite of the very high levels of JunB that presumably arise from constitutive activation of nuclear factor-κB (NF-κB), JunB is transcriptionally inactive in Hodgkin lymphoma.

These observations again support our conclusion, namely that high levels of transcriptionally active JunB are incompatible with B-lymphoid transformation.

We have not systematically investigated all possible mechanisms that may underlie the escape. However, in several cell lines derived from explanted murine tumors, we observed a loss of p16 expression, which is a target gene for JunB. This loss is per se sufficient to account for the escape of the murine v-abl-transformed B cells from JunB-mediated growth inhibition. A loss
of p16 leads to deregulation of cyclin D–dependent kinase activity and thus promotes progression through G1.3144 However, there are various possibilities to overcome growth inhibition by p16 and alterations of cyclin D–dependent complexes are reported in more than 90% of all human lymphomas.4546 One of the underlying molecular mechanisms is indeed loss of p16 protein expression due to hypermethylation of cytosine-phosphate-guanosine (CpG) islands.46 Additional escape mechanisms are likely to exist. Promoter methylation of JunB was shown to be a mechanism to down-regulate JunB in human CML cells.25 Taken together, both the earlier observations and the current findings highlight the tumor-suppressing role of JunB in B-lymphoid cells. In all murine v-abl–transformed cell lines we investigated, the ability of JunB to induce elevated levels of p16 was lost, indicating the loss of a normal cell cycle control in these cells.

Given the various escape mechanisms that are likely to be operative at the time point of diagnosis, it is at present, however, difficult to predict the prognostic role of JunB.

Acknowledgments

We are indebted to Latifa Bakiri and Peter Valent for helpful comments and critical reading of the manuscript. We are grateful to H. Drobny and R. Liebinger for technical help and to N. Rosenberg and M. Yaniv for sharing of reagents. We also want to thank U. Losert and the staff of the Institute for Biomedical Research at the Vienna University Clinics (AKH) for the good collaboration in maintaining our mouse colony.

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