Genome-wide RNAi screen identifies suppressors of BCR-ABL-induced leukemia

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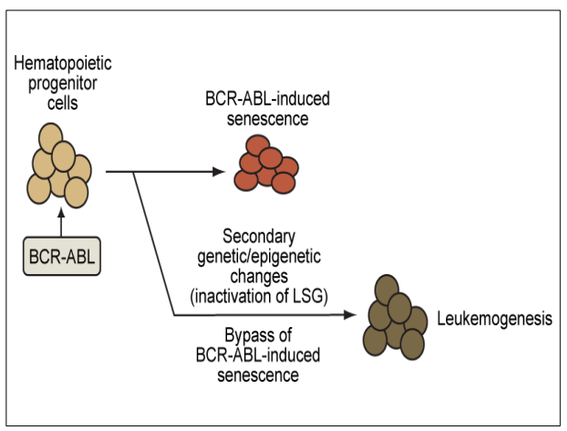
ABSTRACT

Certain oncogenes that promote solid tumors, such as *RAS* and *BRAF*, induce senescence when expressed in primary cells. Oncogene-induced senescence plays an important role in suppressing tumorigenesis by preventing proliferation of cells at risk for neoplastic transformation. Thus, in many instances, genes involved in oncogene-induced senescence, such as *TP53* and *RB1*, are also tumor suppressors. BCR-ABL is an oncogenic kinase derived from the translocation between chromosomes 9 and 22 that can transform myeloid progenitor cells and drives the development of the vast majority of chronic myeloid leukemia (CML) cases and 20%–30% of adult acute lymphoblastic leukemia (ALL) cases. We have recently found that BCR-ABL, as well as two other leukemogenic fusion-proteins, CBFB-MYH11 and RUNX1-ETO, can, like RAS and BRAF, induce senescence in primary human fibroblasts and hematopoietic progenitors. Our results imply that the development of BCR-ABL+ leukemias involves genetic and/or epigenetic alterations that inactivate one or more senescence-promoting genes. Consistent with this hypothesis, inactivation of genes known to promote senescence, such as *TP53* and *CDKN2A* (*p16^{INK4a}/p14^{ARF}*), can cooperate with BCR-ABL in mouse models of CML, and *CDKN2A* deletions are frequently found in patients with BCR-ABL+ ALL. Significantly, these same genetic alterations can also confer resistance to imatinib, an inhibitor of BCR-ABL kinase activity that is the first-line treatment for CML. Thus, delineating the genes and regulatory pathways by which BCR-ABL induces senescence in primary cells will help identify genetic alterations that cooperate with BCR-ABL to promote leukemogenesis and are responsible for the emergence of imatinib resistance.

HYPOTHESIS

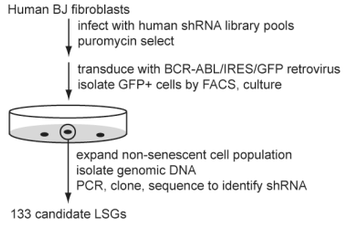
Project Hypothesis

We propose the existence of genes that are involved in BCR-ABL-induced senescence that normally suppress leukemogenesis. Inactivation of these genes cooperates with BCR-ABL to induce leukemia, accelerate disease progression and/or confer drug resistance.

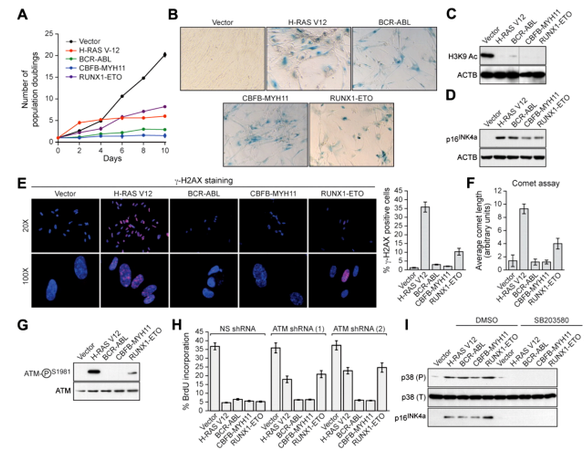


METHODS

To identify genes required for BCR-ABL-induced senescence, we performed a large-scale RNAi screen that is summarized in Figure below. We used The RNAi Consortium (TRC) shRNA library from Open Biosystems, which comprises 85,000 shRNAs targeting ~15,000 human genes. We screened the entire shRNA library by analyzing a series of pools, each of which contained 5000 shRNAs. A non-silencing (NS) shRNA was analyzed in parallel to assess background. We performed the screen in primary human fibroblasts, rather than hematopoietic progenitors (the cell type most relevant to BCR-ABL+ leukemia), for two main reasons. First, fibroblasts are more amenable to large-scale screening than hematopoietic progenitors. Second, our previous study revealed important similarities between BCR-ABL-induced senescence in primary fibroblasts and hematopoietic progenitors, including common signaling pathways required for senescence induction.

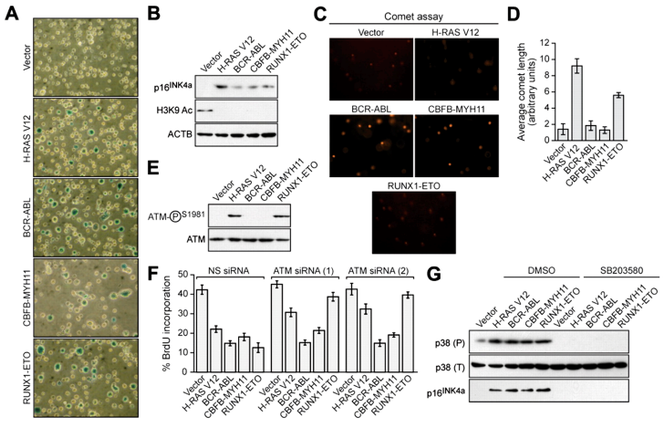


RESULTS

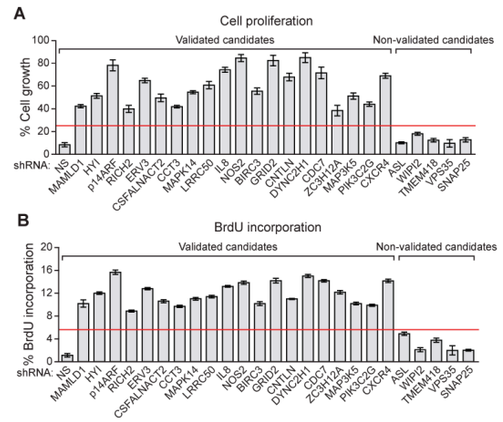


Leukemogenic fusion proteins induce senescence in human primary fibroblasts. (**A**) IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein were monitored for proliferation by trypan blue viability assay. (**B**) IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein were stained for senescence-associated beta-galactosidase. (**C**) Immunoblot analysis monitoring H3K9 acetylation (H3K9 Ac) in IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**D**) Immunoblot analysis monitoring p16INK4a levels in IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**E**) IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein were stained for gamma-H2AX and analyzed by fluorescence microscopy. (Right) Quantification of gamma-H2AX staining. Cells with more than 10 gamma-H2AX foci were included in the analysis. (**F**) IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein were analyzed by comet assay under alkali conditions, and comet tail length was quantified. (**G**) Immunoblot analysis monitoring levels of phospho-ATM and, as a control, total ATM in IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**H**) BrdU incorporation assay in IMR90 cells stably expressing either a nonsilencing (NS) shRNA or one of 2 unrelated shRNAs directed against ATM, and expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**I**) Immunoblot analysis of phosphorylated p38 (P), total p38 (T), and p16INK4a levels in IMR90 cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein treated with dimethyl sulfoxide (DMSO) or the p38 MAPK inhibitor SB20358. Error bars represent Standard error mean (SEM).

RESULTS



Leukemogenic fusion proteins induce senescence in hematopoietic progenitors. (**A**) Bone marrow cells were isolated from 5-fluorouracil-treated mice, transduced with a retrovirus expressing empty vector, H-RAS V12, or a leukemogenic fusion protein, and stained for senescence-associated beta-galactosidase. (**B**) Immunoblot analysis monitoring levels of p16INK4a and H3K9Ac in hematopoietic cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**C**) Hematopoietic cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein were analyzed by comet assay under alkali conditions. (**D**) Quantification of comet tail length. Error bars represent SEM. (**E**) Immunoblot analysis monitoring levels of phospho-ATM and, as a control, total ATM in hematopoietic cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. (**F**) BrdU incorporation assay in hematopoietic cells stably expressing either a nonsilencing (NS) siRNA or one of 2 unrelated siRNAs directed against ATM, and expressing empty vector, H-RAS V12, or a leukemogenic fusion protein. Error bars represent SEM. (**G**) Immunoblot analysis of phosphorylated p39 (P), total p38 (T), and p16INK4a levels in hematopoietic cells expressing empty vector, H-RAS V12, or a leukemogenic fusion protein treated with dimethyl sulfoxide (DMSO) or SB203580.



D

Gene	Percent of BCR-ABL+ ALL samples with decreased copy number
CDKN2A	48.8% (21/43)
CCT6A	23.3% (10/43)
CNTLN	20.9% (9/43)
WIPI2	20.9% (9/43)
ASL	16.3% (7/43)
BCAP29	16.3% (7/43)
ERV3	16.3% (7/43)
TYW1	16.3% (7/43)
PIP5KL1	14.0% (6/43)
FCN1	11.6% (5/43)
OR1N2	11.6% (5/43)
TXN	11.6% (5/43)

Validation of a subset of randomly selected candidates. (**A**) Cell proliferation assay. The red cut-off line indicates a 3-fold increase over NS. (**B**) BrdU incorporation assay. The red cut-off line indicates a 5-fold increase above NS. Error bars indicate SEM (**C**) List of candidate Leukemia suppressor genes (LSGs) with significantly decreased copy number in BCR-ABL+ ALL. (**D**) Identification of eight candidate LSGs that are significantly down-regulated in blast crisis compared to chronic phase CML. For all genes shown, p<1e-11.

CONCLUSIONS

1. Leukemogenic fusion proteins (BCR-ABL, CBFB-MYH11, RUNX-ETO) induce senescence in both primary human fibroblasts and hematopoietic progenitors.
2. Leukemogenic fusion proteins induce senescence by activating p38 MAP Kinase pathway and blocking this pathway abrogates their ability to induce senescence.
3. Senescence induction by leukemogenic fusion proteins appears to be one of the major tumor suppressive pathway in hematopoietic progenitors.
4. Our genome-wide RNAi screen has identified 104 genes that upon shRNA-mediated knockdown bypass BCR-ABL-induced senescence.
5. A subset of genes identified in the RNAi screen have been found to be deregulated in CML and ALL patient samples, which indicates that inhibition of cellular senescence pathway by leukemogenic proteins contributes to the development of leukemia.
6. Reactivation of cellular senescence pathway can be explored as a therapeutic strategy to treat leukemogenic fusion protein-driven leukemia.

REFERENCES

1. Wajapeyee N, Wang SZ, Serra RW, Solomon, PD, Nagarajan, A, Zhu X, and Green MR (2010). Senescence induction in human fibroblasts and hematopoietic progenitors by leukemogenic fusion proteins. Blood 115: 5057-60.
2. Williams RT, Roussel MF, Sherr CJ. Arf gene loss enhances oncogenicity and limits imatinib response in mouse models of Bcr-Abl-induced acute lymphoblastic leukemia. PNAS, 2006 Apr 25; 103(17):6688-93.
3. Wendel et al., Loss of p53 impedes the antileukemic response to BCR-ABL inhibition. PNAS, May 9, 103(19): 7444-9.
4. Mulligan et al., Genomic analysis of clonal origins of relapsed acute lymphoblastic leukemia. Science. 2008 Nov 28, 322 (5906):1377-80.
5. Mulligan et al., BCR-ABL1 lymphoblastic leukemia is characterized by the deletion of Ikaros. Nature. 2008 May 1; 453(7191):110-4.
6. Radich et al., Gene expression changes associated with progression and response in chronic myeloid leukemia. PNAS. 2006 103 (8) 2794-2799.

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