Oncogenic NRAS, KRAS, and HRAS Exhibit Different Leukemogenic Potentials in Mice

Chaitali Parikh,1 Ramesh Subrahmanyam,1,2 and Ruibao Ren1

1Rosenstiel Basic Medical Sciences Research Center, Department of Biology and 2Graduate Program in Biophysics and Structural Biology, Department of Biochemistry, Brandeis University, Waltham, Massachusetts

Abstract

RAS proteins are small GTPases that play a central role in transducing signals that regulate cell proliferation, survival, and differentiation. The RAS proteins interact with a common set of activators and effectors; however, they associate with different microdomains of the plasma membrane as well as other endomembranes and are capable of generating distinct signal outputs. Mutations that result in constitutive activation of RAS proteins are associated with ~30% of all human cancers; however, different RAS oncogenes are preferentially associated with different types of human cancer. In myeloid malignancies, NRAS mutations are more frequent than KRAS mutations, whereas HRAS mutations are rare. The mechanism underlying the different frequencies of RAS isoforms mutated in myeloid leukemia is not known. In this study, we compared the leukemogenic potential of activated NRAS, KRAS, and HRAS in the same bone marrow transduction/transplantation model system. We found that all three RAS oncogenes have the ability to induce myeloid leukemias, yet have distinct leukemogenic strengths and phenotypes. The models established here provide a system for further studying the molecular mechanisms in the pathogenesis of myeloid malignancies and for testing targeted therapies. [Cancer Res 2007;67(15):7139-46]

Introduction

RAS proteins are small GTPases that act as molecular switches to transduce signals from activated receptors. They do so by cycling between a GDP-bound inactive state and a GTP-bound active state. When in its GTP-bound state, RAS can bind to and activate a range of downstream effector proteins, which may then result in diverse cellular outcomes like cell proliferation, survival, differentiation, and neoplastic transformation (reviewed in refs. 1, 2).

Three RAS genes code for four highly homologous RAS proteins, NRAS, HRAS, and KRAS4B/KRAS4A ( splice variants). These proteins have identical effector binding domains and hence can interact with the same set of downstream effectors. However, due to differences in their posttranslational modifications, they have different trafficking routes and localize to distinct microdomains of the plasma membrane and other endomembranes (3). As a result of this, they may have access to different effector pools and may be capable of generating distinct signal outputs (4). Indeed, RAS isoforms have been shown to differ in their abilities to activate various downstream proteins (5–7). Oncogenic versions of HRAS are better than NRAS or KRAS at transforming fibroblast cells, whereas NRAS is better at transforming hematopoietic cells (8). Gene knockout studies further highlight these differences. Knocking out NRAS or HRAS or both in mice results in essentially normal adults, whereas KRAS-deficient mice are embryonic lethal (9, 10).

Nearly 30% of human cancers, including solid tumors and hematologic malignancies, are associated with mutations in RAS genes. Interestingly, mutations in different RAS isoforms are preferentially associated with cancers of different organs (11). For example, KRAS mutations are found in nearly 90% of pancreatic cancers. In myeloid malignancies, NRAS mutations are more frequent than KRAS mutations, whereas HRAS mutations are rare. The mechanism underlying the different frequencies of RAS isoforms mutated in myeloid malignancies is not known.

The leukemogenic potential of oncogenic RAS has been studied in animals by transgenic as well as bone marrow transduction/transplantation (BMT) models. Transgenic mice expressing HRAS under the mouse mammary tumor virus promoter/enhancer developed B-lymphoblastic leukemia, whereas expression of HRAS in a BMT model induced B and T lymphoid leukemia/lymphoma (12, 13). Transgenic mice expressing NRAS under the IgH E enhancer or the hMRP8 promoter developed T lymphoid leukemias or epithelial tumors (14, 15). Expression of NRAS under the Moloney murine leukemia virus long terminal repeat (Mo-MuLV LTR) in a BMT model induced myeloid malignancies with a long latency and incomplete penetrance (16). These studies suggested that activation of RAS by itself might not be sufficient to induce myeloid leukemias. However, recently, others and we have shown that expression of activated mutants of NRAS and KRAS can efficiently induce myeloid leukemias in mice (17–19). Expression of oncogenic NRAS using a BMT model induces an acute myeloid leukemia (AML)– or chronic myelomonocytic leukemia (CMML)–like disease in mice, whereas expression of oncogenic KRAS under its endogenous promoter in a conditional knock-in strain gives rise to a CMML-like disease in all the mice. Because oncogenic RAS proteins were studied in different model systems, it is not clear whether the difference in phenotypes of RAS oncogenes is due to different methods used to express the oncogenes or due to differences in their intrinsic leukemogenic potentials. Given that RAS proteins have both shared and distinct biochemical and biological functions, direct comparison of their leukemogenic potentials could provide insights into the mechanism of RAS leukemogenesis and help to identify critical targets of RAS for developing therapies.

In this study, we sought to compare NRAS, KRAS, and HRAS leukemogenesis by expressing them in the same model system. We find that all NRAS, KRAS, and HRAS have the potential to induce myeloid leukemia in mice, but differ in terms of their potency and disease phenotype.
Materials and Methods

**DNA constructs.** The NRASD12, KRASD12, and HRASV12 genes were amplified by PCR from expression sequence tags for human NRAS (Genbank accession no. NM_002255) and HRAS (Genbank accession no. AF182383 and AF149387) and cDNA for KRAS (Genbank accession no. AF039171; UMR cDNA Resource Center). The activated mutants of NRAS, KRAS, and HRAS were generated by PCR, by introducing mutations in codon 12 of these genes, which changed glycine to aspartic acid (G12D) in NRAS and KRAS and into valine (G12V) in HRAS. The changes in codon 12 were GGT to GAT for NRASD12 and KRASD12 and GCC to GTC for HRASV12. The genes were cloned into 5′-NotI and 3′-ClaI restriction enzyme sites in the murine stem cell virus (MSCV) vector, downstream of an internal ribosomal entry site (IRES) from the encephalomyocarditis virus (EMCV). All NRASD12, KRASD12, and HRASV12 were expressed as myc-tagged proteins downstream of the IRES element, whereas green fluorescent protein (GFP) was expressed under the control of the MSCV LTR. The control vector, MSCV-IRES-GFP (MIG) consists of only the GFP gene downstream of the EMCV-IRES. The fidelity of PCR cloning was confirmed by sequencing.

**Retrovirus production and determination of viral titer.** Bosc23 cells, a retroviral packaging cell line, were used for producing retroviruses used for infecting cell lines as well as primary bone marrow as previously described (20). Briefly, 2 x 10^6 Bosc23 cells were plated −12 h before transfection. Five micrograms of the DNA of interest were transfected into the Bosc23 cells by the calcium phosphate precipitation method. The transfection medium was replaced with fresh medium −10 h posttransfection. The viral supernatant from the transfected cells was collected 48 h after transfection. The viral titer was calculated in transducing units (TU) by multiplying the percentage of NIH3T3 cells expressing GFP and the number of cells seeded. The titers in this experiment were −4 x 10^7 TU/mL for NRAS, KRAS, and HRAS (matched) and −5 x 10^6 TU/mL for KRAS-high titer condition and vector control.

**Bone marrow transduction/transplantation.** Bone marrow cells were isolated from 6- to 8-week-old, 5-fluorouracil (5-FU; 250 mg/kg) treated, donor BALB/c mice (Taconic Farms). They were infected with retroviruses, and cultured for 2 days in vitro, in the presence of cytokines. They were then transplanted, by injecting 4 x 10^6 cells, into the tail vein of each of the lethally irradiated (2 x 450 rad, 3 hours each dose) female recipient BALB/c mice as described (21). The recipient mice were monitored for signs of disease from day 14 posttransplantation.

**Flow cytometry analysis.** Peripheral blood cells or bone marrow cells (0.5–1 million) were used for staining with each of the following antibodies, either alone or in combination, after blocking with purified anti-mouse CD16/CD32 (2.4G2, BD PharMingen). They were incubated with retroviruses, and then washed, in the presence of cytokines. They were then transplanted, by injecting 4 x 10^6 cells, into the tail vein of each of the lethally irradiated (2 x 450 rad, 3 hours each dose) female recipient BALB/c mice as described (21). The recipient mice were monitored for signs of disease from day 14 posttransplantation.

**Western blot analysis.** Cell lysates were prepared from infected NIH3T3 cells and from ACK-treated single-cell suspensions of mouse tissues by adding equal volume of 2× Laemmli sample buffer to the cell suspensions in PBS. For assays using 32Dcl3 cells, lysates were prepared after withdrawing interleukin 3 for 12 h. The lysates were heated at 100°C for 5 min and centrifuged to remove debris. Lysates were then resolved on 5%–12% polyacrylamide gels, transferred to nitrocellulose membranes, and blotted with the following primary antibodies: anti-Ras (RAS10, Upstate Biotechnology); anti-actin (AC40, Sigma); anti-myc tag 9E10 monoclonal antibody (from conditional medium of 9E10 hybridoma cell line); anti-dynamin (BD Biosciences); and pAkt, Akt, pMek1/2, Mek1/2, pErk42/44, Erk42/44, pS6er, and S6erp (All 1:1,000, Cell Signaling Technologies). Horseradish peroxidase–labeled goat anti-mouse IgG or goat anti-rabbit IgG (Southern Biotechnology) was used as secondary antibodies. Calculation of ratios of Ras to actin or dynamin was done on a Macintosh computer using the public domain NIH Image program (developed at the NIH and available online).

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Figure 1. Retroviral vectors and Ras expression. A, diagrammatic representation of retroviral vectors used in this study. Bicistronic vectors were used that could coexpress Ras and GFP. Vector control expresses GFP alone. Positions of restriction enzyme sites as well as probe used for Southern blot are indicated. B, Western blot analysis of lysates prepared from NIH3T3 cells infected with NRAS, KRAS, and HRAS retroviruses, as well as the vector control (lanes 1, 2, 3, and 4, respectively) using anti-panRas and Myc-tag antibodies. Detection of dynamin using an anti-dynamin antibody was used as a loading control.

Zeta-probe GT membrane (Bio-Rad). The membrane was hybridized with a 32P-labeled 0.7 kb NcoI-SalI fragment corresponding to GFP as a probe. The blot was stripped with 0.1% boiling SDS solution and reprobed with 32P-labeled 1.4 kb if-4 DNA fragment as a loading control. To generate a control for single-copy proviral integration, we did single-cell sorting of 32D cells transduced with MSCV-BCR/ABL-IRES-GFP. We then tested the single-cell clones by Southern blotting, and isolated a cell line with a single-copy provirus (Fig. 5A, lane 1).

**Western blot analysis.** Cell lysates were prepared from infected NIH3T3 cells and from ACK-treated single-cell suspensions of mouse tissues by adding equal volume of 2× Laemmli sample buffer to the cell suspensions in PBS. For assays using 32Dcl3 cells, lysates were prepared after withdrawing interleukin 3 for 12 h. The lysates were heated at 100°C for 5 min and centrifuged to remove debris. Lysates were then resolved on 5%–18% gradient polyacrylamide gels, transferred to nitrocellulose membranes, and blotted with the following primary antibodies: anti-Ras (RAS10, Upstate Biotechnology); anti-actin (AC40, Sigma); anti-myc tag 9E10 monoclonal antibody (from conditional medium of 9E10 hybridoma cell line); anti-dynamin (BD Biosciences); and pAkt, Akt, pMek1/2, Mek1/2, pErk42/44, Erk42/44, pS6er, and S6erp (All 1:1,000, Cell Signaling Technologies). Horseradish peroxidase–labeled goat anti-mouse IgG or goat anti-rabbit IgG (Southern Biotechnology) was used as secondary antibodies. Calculation of ratios of Ras to actin or dynamin was done on a Macintosh computer using the public domain NIH Image program (developed at the NIH and available online).
Results

Oncogenic NRAS, KRAS, and HRAS induce fatal diseases in mice with different latencies. To compare the leukemogenic potentials of activated mutants of NRAS, KRAS, and HRAS, we constructed retroviral vectors expressing myc-tagged NRAS\textsuperscript{D12}, KRAS\textsuperscript{D12}, and HRAS\textsuperscript{V12} (D12 and V12 mutations were chosen based on their being most frequent mutations found in the corresponding RAS genes; Fig. 1A). The expression of the activated RAS proteins in NIH3T3 cells was confirmed by Western blotting (Fig. 1B). We then infected bone marrow cells isolated from 5-FU–treated mice with retroviruses containing NRAS, KRAS, or HRAS or vector alone and transplanted these cells into lethally irradiated syngeneic recipient mice. 5-FU is a pyrimidine analogue that induces cell cycle arrest and apoptosis by inhibiting the ability of the cell to synthesize DNA (22). Its role here is to eliminate the proliferating hematopoietic precursor cells and to enrich and stimulate hematopoietic stem cells.

Using this system, we have shown that activated NRAS rapidly and efficiently induces a CMML- or AML-like disease in mice (19). In this study, NRAS is used as a control and the data presented here are from the new experiment where we compare NRAS, KRAS, and HRAS leukemogenesis. We found that oncogenic mutants of all three RAS isoforms are capable of inducing fatal diseases in mice. Under the same experimental conditions (same retroviral titers and same pool of donor bone marrow cells), the disease latency of HRAS\textsuperscript{V12} mice was significantly shorter than NRAS\textsuperscript{D12} and KRAS\textsuperscript{D12} mice (P < 0.001 and P < 0.0001, respectively). Although the majority of the NRAS\textsuperscript{D12} mice died faster than KRAS\textsuperscript{D12} mice, the difference in their disease latencies was not statistically significant (P = 0.2; Fig. 2; Table 1). All vector control mice remained healthy during the course of the experiment.

Activated NRAS, KRAS, and HRAS induce myeloid leukemias with distinct phenotypes. We sacrificed moribund mice to determine the disease phenotype in each case. As shown previously, the mice receiving NRAS\textsuperscript{D12} transduced bone marrow cells succumbed to either of two diseases. Some mice developed leukocytosis, anemia, enlarged spleens, and livers. The leukemic cells were predominantly mature granulocytes and monocytes. This disease resembled human CMML. The other disease was characterized by the presence of immature myeloid cells, severe anemia and hepatosplenomegaly, and resembled human AML (19). The frequencies of these different diseases seemed to correlate with the

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<th>Construct</th>
<th>No. animals</th>
<th>Disease</th>
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<th>WBCs, $10^3$/μL</th>
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\* Median latency within the particular group.
\textsuperscript{1} WBC count is given as the range of WBCs for the diseased mice within a particular disease group.
\textsuperscript{2} Hematocrit, liver, and spleen weights are average ± SD.
\textsuperscript{3} Mice transduced with vector control, MSCV-IRES-GFP (MIG).
\textsuperscript{4} Not applicable.
\textsuperscript{5} High titer.

Figure 2. Cumulative survival of oncogenic NRAS, KRAS, and HRAS mice. Cumulative survival curves of mice transplanted with oncogenic NRAS, KRAS, and HRAS or vector control (MIG) transduced bone marrow cells were generated by Kaplan-Meier survival analysis. Donor bone marrow cells were transduced under closely matched titers of NRAS, KRAS, and HRAS retrovirus: $\sim 4 \times 10^7$ TU/mL for all RAS and $5 \times 10^6$ TU/mL for KRASD12 high titer (HT) and vector control.
retroviral titers used, with higher titers favoring AML (Fig. 3 and data not shown).

Analysis of the peripheral blood, bone marrow, livers, and spleens of all diseased KRASD12 mice by smears and touch preparations revealed the accumulation of mainly mature myeloid cells. Flow cytometry analysis of peripheral WBC showed two major populations. One expressed the granulocytic surface markers (Mac-1+, Gr-1hi, CD16/32+) and the other expressed monocytic cell surface markers (Mac-1’, Gr-1lo/−, F4/80+, M/CSFR’, Moma-2’, CD16/32’, CD31’, CD86’, and CD38’; Fig. 3). The level of

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Figure 3. Immunophenotyping of leukemic cells from diseased oncogenic NRAS, KRAS, and HRAS mice. Bone marrow or peripheral blood cells were isolated from diseased mice, stained with antibodies to various cell surface markers as indicated, and subjected to flow cytometry analysis. GFP expression is along the X axis, and Y axis shows expression of the cell surface marker specified over each column.
leukocytosis varied among the mice, with the majority developing WBC counts higher than 50,000/μL, but some with counts between 10,000/μL and 50,000/μL. However, even in these mice, most cells were mature myeloid cells. These mice also develop anemia and have enlarged livers and spleens due to leukemic infiltration and extramedullary hematopoiesis (Table 1). This disease is similar to the CMML-like disease seen in some NRASD12 mice.

Because mice expressing NRASD12 develop either an AML-like or a CMML-like disease that correlate with retroviral titers, we wanted to check if this was true for KRASD12. We therefore conducted the experiment under different titer conditions, ranging from extremely high titers to much lower titers and found that the disease latency does correlate with the retroviral titer used—mice receiving higher titers succumb to disease faster than those receiving lower titers of the same virus (Fig. 2 and data not shown). However, unlike oncogenic NRAS, all KRAS mice succumbed to a similar CMML-like disease regardless of the titers used (Table 1 and data not shown).

Analysis of peripheral blood, bone marrow, livers, and spleens from HRASV12 mice revealed the abnormal presence of immature myeloid cells. Flow cytometry analyses show that the majority of the GFP-positive cells are Mac-1⁺ CD38⁺ and CD16/32⁺ but Gr-1⁻/-, M/CSFR-/-, and MOMA-2-⁻. A fraction of the leukemic cells also express CD117 (c-Kit), CD34, Thy1.2, F4/80, CD86, and CD31, indicating a mixed blast and differentiated monocytic population (Fig. 3). These mice also develop severe anemia, as well as massively enlarged livers and spleens (Table 1). This disease resembles the AML-like disease seen in some NRASD12 mice. Importantly, when we did experiments using lower titers of HRAS, we found that all the mice still succumbed to an AML-like disease (data not shown).

Although both NRAS and HRAS induce an AML-like disease in mice, the invasiveness of the two diseases is drastically different. NRASD12 mice showed some leukemic infiltration in the lung tissue, but mice receiving HRASV12 showed massive infiltration of lungs with leukemic cells, along with widespread pulmonary hemorrhages (Fig. 4). This high invasiveness of the tumor correlates with the shorter disease latency of HRAS mice.

In addition to these predominant phenotypes, majority of the KRAS and the HRAS mice (similar to the NRAS mice) have increased mast cells in the peripheral blood, bone marrow, livers, and spleens. This indicates that all oncogenic RAS mutants can also induce mastocytosis in mice.

**NRAS-, KRAS-, and HRAS-induced myeloid leukemias are oligoclonal.** To further compare the leukemogenic potentials of activated mutants of RAS isoforms, we examined the clonality of the leukemias by Southern blot analysis (Fig. 5). Restriction enzyme BglII, which recognizes a unique site in the RAS proviral DNA, was used to check proviral integration, and XbaI, which cleaves the RAS proviral DNA at the LTRs, was used to check the integrity and the total amount of proviruses (Fig. 1A). Genomic DNA isolated from a cloned MSCV-BCR/ABL-IRES-GFP–infected 32D cell line was used as a single-copy provirus control (Materials and Methods and Fig. 5, lane 1). After probing with 32P-labeled GFP, the blot was stripped and reprobed with a 32P-labeled irf-4 DNA fragment to check for loading. We found that the majority of the leukemias induced by NRAS, KRAS, and HRAS are oligoclonal. This suggests that all oncogenic RAS genes may require secondary oncogenic events to induce leukemias.

**Expression levels of RAS and activation of RAS downstream signaling pathways by oncogenic NRAS, KRAS, and HRAS.** To gain insights into the mechanism underlying different phenotypes of the RAS isoforms, we compared the signaling pathway activation by the oncogenic RAS proteins. We infected 32Dc13 cells, a murine myeloid progenitor cell line, with retroviral constructs expressing activated NRAS, KRAS, and HRAS and sorted for GFP-positive cells to generate nearly pure cell lines. As controls, we generated cell lines expressing GFP alone (empty vector) or the BCR/ABL oncogene. We then checked for the activation of signaling proteins Mek, Erk, Akt, PDK1, mTOR, and phospho-S6 ribosomal protein (pS6rp; Fig. 6A). We found that none of the RAS isoforms activated the mitogen-activated protein kinase pathway above basal levels (as evidenced by phosphorylation of Mek and Erk) in 32D cells.
whereas BCR/ABL did. In contrast, oncogenic NRAS and HRAS showed increased phosphorylation of components of the phosphatidylinositol 3-kinase pathway (pPDK1, pAkt, pMTOR, pS6rp), whereas KRAS is a much weaker activator, if at all, of this pathway. It is possible that this differential activation of the phosphatidylinositol 3-kinase pathway underlies the phenotypic differences among the RAS isoforms.

Next, we checked for levels of expression of exogenous RAS in these cell lines. We observed that in myeloid progenitor cells, the expression levels of RAS proteins are drastically different, with HRAS expression the highest and KRAS the lowest (Fig. 6B). The lower expression level of KRAS may account for, at least partially, the reduced activation of the phosphatidylinositol 3-kinase pathway in KRAS-expressing 32D cells.

We have previously shown that overexpression of oncogenic NRAS in leukemic cells may contribute to the development of AML versus CMML. We went on to test whether differential expression of oncogenic RAS also correlates with the phenotypic differences among the NRAS, KRAS, and HRAS mice. We checked levels of RAS expression in tumors from NRAS, KRAS, and HRAS mice and found that, in general, RAS proteins are expressed at higher levels in AML (HRAS and NRAS) than CMML (NRAS and KRAS; Fig. 6C).

Discussion

In this study, we have compared the leukemogenic potential of oncogenic NRAS, KRAS, and HRAS in the same BMT model system. We found that although all three RAS isoforms have the potential
to induce myeloid leukemias in mice, they do so at different rates and produce distinct phenotypes. Previously, we have modeled NRAS leukemogenesis using the same system and shown that oncogenic NRAS can induce an AML- or CMML-like disease in mice. Here, we show that oncogenic HRAS always induces an AML-like disease, whereas oncogenic KRAS invariably induces a CMML-like disease in mice.

Expression of oncogenic KRAS in a conditional knock-in mouse strain has been shown to exclusively induce a CMML-like disease (17, 18). The result that oncogenic HRAS always induces an AML-like disease, whereas oncogenic KRAS invariably induces a CMML-like disease in mice.

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Interestingly, the KRAS protein level is lower than NRAS and HRAS in myeloid cells, although the same expression vector was used for all three RAS oncogenes and they expressed equally in NIH3T3 fibroblast cells (Fig. 1B). One possibility is that KRAS is regulated posttranslationally in hematopoietic cells. Alternatively, high expression of KRAS might activate signaling pathways that negatively regulate cell proliferation and/or survival. Further study of this unique property of KRAS may help to develop therapies for KRAS-positive malignancies.

HRAS mutations are rare in human hematologic malignancies. Recent findings show that patients with germ line mutations in HRAS are not particularly predisposed to leukemias (24). However, we found in this study that activated HRAS can be a potent oncogene in the induction of myeloid leukemia. This seeming discrepancy could be due to differences in regulation of gene expression. Retrovirus-mediated transgene expression varies in target cells depending on its site of integration in the host genome. The combination of certain integration sites and the strong retroviral LTR promoter/enhancer can result in very high expression of the transgene in target cells. Indeed, we found that KRAS leukemic cells express high levels of GFP and HRAS (Figs. 3 and 6). In contrast, HRAS expression is low in normal hematopoietic cells—the lowest among the three RAS genes (25). It is, therefore, possible that HRAS has the potential to induce myeloid malignancies when it is overexpressed, but normally its expression is tightly regulated in hematopoietic cells, making it difficult for cells with HRAS mutations to develop into tumors. Consistent with this idea, overexpression of human HRAS was found to be responsible for tumors induced by chemical carcinogens in mice (26). These observations suggest that overexpression of HRAS, in addition to its oncogenic mutation, plays an important role in tumorigenesis. However, we cannot rule out the possibility that the different oncogenic potential of HRAS in human versus mouse could be due to species-specific differences.

We have previously shown that oncogenic NRAS induced AML-like or CMML-like disease seems to correlate with the level of NRAS expression, with high expression favoring AML and lower expression correlating with CMML. We found here that this correlation extends to all RAS oncogenes (Fig. 6). This result
suggests that overexpression of RAS oncproteins also plays an important role in the pathogenesis of AML. Consistent with this idea, overexpression of wild-type and oncogenic RAS is indeed common in human AML (27, 28).

It has been shown that overexpression of oncogenic RAS in fibroblast and epithelial cells cause senescence or apoptosis (29). We show here that all oncogenic RAS proteins can efficiently induce myeloid malignancies. This highlights the fact that the outcome of RAS signaling is very dependent on cellular context. It is possible that myeloid cells contain factors that can overcome the negative signaling activated by RAS oncogenes. Consistently, c-Myc has been shown to require a second oncogenic hit to suppress apoptosis in inducing lymphoid but not myeloid leukemia (30). Identifying factors that permit RAS transformation in myeloid cells may help to develop therapies for human myeloid malignancies.

The Raf/Mek/Erk pathway was the first downstream signaling pathway of RAS to be identified and it is required for RAS transformation of fibroblast cells (31). However, we found here that activation of Mek and Erk was not detected in 32D myeloid progenitor cells expressing any one of the three RAS oncogenes (Fig. 6). This result is consistent with the finding that there is no activation of Mek and Erk in leukemic cells from the oncogenic KRAS conditional knock-in mice (17). These observations suggest that myeloid leukemogenesis by oncogenic RAS may involve unique RAS signaling networks. Identification of critical molecular events in RAS leukemogenesis is important for developing targeted therapies for myeloid malignancies involving RAS. The models established here provide a system to achieve this goal.

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Oncogenic NRAS, KRAS, and HRAS Exhibit Different Leukemogenic Potentials in Mice

Chaitali Parikh, Ramesh Subrahmanyam and Ruibao Ren