Targeting Ras in Myeloid Leukemias

Benjamin S. Braun and Kevin Shannon

Abstract Ras proteins normally relay growth-promoting signals from many activated cell surface receptors, and they are altered by oncogenic point mutations in ~30% of human cancers. Activating KRAS and NRAS mutations are especially common in malignancies of the pancreas, lung, and colon, and in myeloid leukemia. Here, we discuss general strategies for targeting hyperactive Ras signaling in cancer cells with specific reference to myeloid malignancies.

Background Cancer is frequently characterized by mutations that subvert normal mechanisms for controlling survival, proliferation, and differentiation by deregulating cytoplasmic signaling networks. Ras proteins are signal switch molecules that relay growth-promoting signals from various activated cell surface receptors by cycling between active GTP-bound and inactive GDP-bound states (Ras\(^{\text{GTP}}\) and Ras\(^{\text{GDP}}\); see Fig. 1; refs. 1–4). Guanine nucleotide exchange factors, such as SOS1, which are activated as a downstream consequence of ligand binding to growth factor receptors, displace guanine nucleotides from Ras. This is followed by passive guanine nucleotide binding, which effectively results in higher Ras\(^{\text{GTP}}\) levels because GTP is much more abundant than GDP in the cytosol. Ras\(^{\text{GTP}}\) interacts productively with effectors that include Raf proteins (A-Raf, B-Raf, and c-Raf), phosphoinositide-3-OH kinase, and RalGDS to activate downstream kinase cascades (reviewed in refs. 4–7). Signaling is terminated when Ras\(^{\text{GTP}}\) is hydrolyzed to Ras\(^{\text{GDP}}\) through a slow intrinsic GTPase activity, which is markedly accelerated by GTPase-activating proteins. Whereas Fig. 1 presents a simplified view of Ras signaling, recent data indicate extensive biochemical complexity with Ras as a central node in a molecular network that includes many positive and negative components, extensive feedback, crosstalk between linear pathways, and cell type specificity.

Somatic RAS point mutations that introduce amino acid substitutions at codons 12, 13, and 61 are found in ~30% of human cancers. These alleles encode Ras proteins that accumulate in the GTP-bound conformation due to both defective intrinsic GTP hydrolysis and resistance to GTPase-activating proteins. KRAS and NRAS mutations are especially prevalent in cancers of the pancreas, lung, and colon. They are also common in myeloid malignancies, which are clonal disorders of hematopoietic stem and/or progenitor cells that show significant genetic, morphologic, and biological heterogeneity. An increased number of myeloid blasts with suppression of normal hematopoiesis is the hallmark of acute myeloid leukemia (AML), whereas myeloproliferative disorders are associated with over-proliferation of one or more lineages that retain the capacity to differentiate, and myelodysplastic syndrome is characterized by cytopenias and aberrant differentiation. Myeloproliferative disorders and myelodysplastic syndrome frequently progress to AML, which is likely due to the acquisition of cooperating mutations. NRAS or KRAS mutations occur in ~20% of AML specimens (8), and Ras signaling is deregulated by somatic mutations in the genes encoding the FLT3 and c-Kit receptor tyrosine kinases in an additional ~25% to 40% of cases (9–20).

Studies of patients with the monocytic myeloproliferative disorders, juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia, further underscore the role of hyperactive Ras in myeloid leukemogenesis (21, 22). Somatic Ras point mutations are found in ~40% of chronic myelomonocytic leukemia and 30% of JMML cases, respectively. Interestingly, children with neurofibromatosis type 1, a dominant familial cancer syndrome caused germline mutations in the NF1 tumor suppressor gene, are at markedly increased risk of developing JMML with loss of the normal NF1 allele (23–26). The observation that NF1 encodes neurofibromin, a GTPase-activating protein for Ras, strongly implicated hyperactive Ras signaling as the initiating event in JMML (1, 27). This view is supported by recent studies showing that activating mutations in PTPN11, which encodes the protein tyrosine phosphatase SHP-2, occur in ~35% of sporadic JMML (28, 29). SHP-2 potentiates Ras signaling in many systems (30), and RAS, NF1, and PTPN11 mutations are rarely found in the same patient. Together, there is strong genetic evidence that myelomonocytic myeloproliferative disorders are fundamentally diseases of hyperactive Ras signaling.
As a dominant onco-protein that is expressed in many cancers, mutant Ras represents a compelling target for the development of small molecule inhibitors. However, biochemical characteristics of the Ras switch make this approach highly problematic. In contrast to mutated tyrosine kinases such as BCR-Abl and the epidermal growth factor receptor, mutant Ras proteins display defective, rather than increased, enzymatic activity. Developing pharmacologic agents to restore normal enzymatic activity is a daunting proposition that has not been achieved to date. This difficult task is even more formidable in the case of mutant Ras due to structural consequences of substations at codons 12, 13, and 61 (31), which add bulky residues to a confined domain of the protein. It is unlikely that binding of a small molecule will relieve this spatial constraint. Furthermore, even if the alternative approach of inhibiting the interaction of Ras with effector molecules were feasible, there would be a high likelihood of associated systemic toxicity if normal Ras proteins were also affected.

Normal and mutant Ras proteins undergo lipid modifications at the carboxyl terminus for localization to the plasma membrane and signaling function. Based on this observation, it was hoped that inhibition of the enzyme farnesyltransferase would prevent prenylation of oncogenic Ras and therefore inhibit its activity (reviewed in refs. 2, 32). However, the Ras isoforms most commonly involved in cancer, N-Ras and K-Ras4B, have alternative processing pathways that can substitute for farnesylation. This almost certainly explains the disappointing clinical efficacy of farnesyltransferase inhibitors. There is a common misconception that the failure of farnesyltransferase inhibitors in the clinic somehow invalidates oncogenic Ras as a therapeutic target in cancer. In truth, these drugs do not significantly inhibit the processing and membrane localization of either N-Ras or K-Ras4B at doses that are tolerable in vivo.

In light of the problems inherent to directly inhibiting oncogenic Ras, attention has turned toward inhibiting downstream components of Ras signaling pathways such as Raf, MAP/ERK kinase, extracellular signal-regulated kinase,
phosphoinositide-3-OH kinase, Akt, and mammalian target of rapamycin (see Fig. 1). This strategy presumes that a specific biochemical target is a major biological effector of oncogenic Ras activity, that cancer cells require its continued activation for survival, and that inhibition will not cause severe toxicity in normal tissues. Ongoing clinical trials using various small molecule inhibitors are testing this general strategy in myeloid malignancies and other cancers (33).

The need for therapeutic index poses significant challenges for the development of signal transduction inhibitors. In only rare cases will a mutant protein be targeted while sparing the normal isoform; the preference of gefitinib for an uncommon mutant epidermal growth factor receptor in lung cancer may be a rare example of this (34, 35). More commonly, small molecules can be expected to inhibit normal and mutant proteins with similar potency; an example of this is the inhibition of c-Abl and c-Kit by imatinib. When the targets are broadly required physiologically, as is the case for Ras and many of its effectors, toxicity may result.

On the other hand, cancer cells may remodel their signaling networks to become more dependent on these signals than are normal cells. Conceptually, signaling networks are often robust dynamic systems, and oncogenic activation may invoke a compensatory homeostatic response that desensitizes distal components (36). For example, chronic stimulation by oncogenic Ras may cause up-regulation of endogenous proteins that serve to attenuate or terminate signaling. Acutely inhibiting the oncogenic hyperstimulation with a drug would then leave the network dominated by these negative components and reduce the overall signal output to a level that is actually below the normal basal state. This in turn could lead to cell death or differentiation. Indirect evidence of this process has been observed in hematopoietic progenitors expressing oncogenic K-Ras, in which phosphorylation of extracellular signal-regulated kinase and S6 become relatively insensitive to stimulation with the cytokine stem cell factor despite being hypersensitive to stimulation with granulocyte-macrophage colony-stimulating factor (37). Such a mechanism can explain how cancer cells can become dependent on oncogenic signals and how malignant cells treated with a signal transduction inhibitor are preferentially killed, as occurs when normal hematopoietic cells recover in chronic myelogenous leukemia patients who receive imatinib.

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Although hyperactive Ras is a common biochemical lesion in myeloid malignancies, it is not known how disease subtype (e.g., myeloproliferative disorders versus AML) or the specific genetic mechanism that results in hyperactive Ras (e.g., a FLT3 mutation versus a RAS mutation versus NF1 inactivation) might influence clinical response. Recent experience with small molecule inhibitors of mutant FLT3 (38–41) and the transient responses of patients with blast crisis chronic myelogenous leukemia to imatinib indicate that advanced cancers such as AML are less amenable to treatment with single targeted inhibitors than chronic myelogenous leukemia. Similarly, the order in which specific mutations occur during the development and progression of cancer will likely influence responses to targeted agents (42). Along these lines, it will be interesting to determine if inhibitors that target hyperactive Ras signaling will be differentially effective in JMML, where RAS, NF1, and PTPN11 mutations suggest that leukemic growth, versus AML, where RAS and FLT3 mutations are thought to represent cooperating events. It is possible that the multiple genetic changes characteristic of AML qualitatively alter the structure or dynamics of cell signaling networks. If so, it will be helpful to determine how specific leukemia-associated mutations affect the behavior of signaling networks both alone and in combination.

Myeloid malignancies are organized in a hierarchy of cells in which only a small fraction exhibits the capacity for long term self-renewal (43–45). This property is restricted to a “leukemia stem cell” population, which is, by definition, required for maintenance of the malignant clone. Because expression of many signaling proteins change during hematopoietic differentiation (46–50), the effects of a mutant protein may be different in leukemia stem cells than in blast cells. Therefore, the design of inhibitors will need to take into account the behavior of signaling networks in leukemia stem cells specifically. This goal has been impeded by the scarcity of leukemia stem cells and other types of cancer stem cells, and by technical challenges inherent in working with primary cells. These difficulties may be addressed by new technologies, such as multiparameter flow cytometry, which exploits cell surface antigens to identify subsets of cells while simultaneously assessing intracellular levels of cytokine-responsive phosphoproteins (51–53). Although technical hurdles remain, this is a promising strategy for directly interrogating signaling events of leukemia stem cells in the presence of various stimuli and inhibitors (37). Computational methods can infer biochemical network connections directly from such data (54).

Accurately characterizing oncogenic signaling networks will likely require studying primary tumor cells that express mutant proteins at endogenous levels. Many cell culture techniques introduce artifacts that can potentially undermine the biological relevance to primary cancer cells. Strains of genetically engineered mice can address this by providing a ready source of primary cells that can be compared biochemically to genetically identical normal controls. These are proving to be useful for investigating the effects of oncogenic mutations, alone or in combination, on cancer cell signaling. Studies in these model systems should help to establish the fundamental architecture of cancer signaling networks that will inform the analysis of primary human cancer cells. One surprising observation that has already emerged from studies of Ras signaling in genetically engineered mice is that Ras-GTP levels and the activation of downstream kinase cascades are less pronounced than would have been anticipated from experiments done in cultured cell lines (42, 55, 56). Moreover, mutant Ras networks are robustly activated by growth factors and are not constitutively saturated. Together, these findings support the idea that oncogenic Ras is not autonomous but requires extracellular inputs for maximal activity. These data have provocative implications regarding the growth of human cancer cells and suggest that inhibiting targets that are biochemically upstream of oncogenic Ras (e.g., activated growth factor receptors, adapter molecules, or exchange factors) is a potential therapeutic strategy.

The rationale for developing cancer therapies that counter the effects of RAS mutations remains compelling 35 years after the discovery of these oncogenes. The biomedical research community and pharmaceutical industry have compiled extensive genetic data from human cancers, identified many components of Ras-related signaling networks, and developed inhibitors of key signaling molecules. Further advances will yield a more sophisticated understanding of how this critical
biochemical network responds dynamically to stimuli, how it behaves in cancer stem cells, how it is modulated by cellular responses and cooperating mutations, and how it regulates effectors of cell fate. Hopefully, these insights will be a cornerstone for developing therapeutic strategies that exploit inherent differences between normal and malignant signaling pathways and thereby improve the therapeutic index of current cancer treatments.

References
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