The discovery of oncogenes provided insight into the molecular underpinnings of cancer and suggested the promise of novel molecular strategies for cancer treatment as highlighted in this issue by Yaari et al. and by Bishop previously (1, 2). However, only recently have effective drugs that target oncogenes been successfully introduced into the clinical setting. The flagship example of a targeted therapeutic is Gleevec (imatinib), which targets the BCR-ABL, c-Kit, and the platelet-derived growth factor receptor oncoproteins as well as other tyrosine kinases. Gleevec has been proven effective in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumor (3, 4). Why has it been so difficult to discover drugs that target oncogenes to treat cancer? Does the discovery of Gleevec suggest that we are on the verge of developing many new drugs for the treatment of cancer?

The difficulty in identifying drugs that target oncogenes for the treatment of cancer is multifactorial. On a theoretical level, most cancers are likely the consequences of many genetic lesions, so the repair or inactivation of one associated mutant gene product may not sufficiently affect the pathobiology of a cancer to have clinical utility. For most tumors, the best oncogenes to target have yet to be identified. Even if a targeted therapy succeeds in having a clinical effect, tumors are genomically unstable and may easily acquire compensatory genetic lesions. Even if certain oncogenes are good targets, it is not clear how they would be identified. Even if they are good targets, many oncogenes are not readily “drugable” using small molecules. Even if drugable, many oncogenes serve essential cellular functions, and sufficiently targeting a mutant oncogene may also inactivate the normal proto-oncogene, thereby causing high toxicity.

Regardless of the various theoretical and practical limitations to targeting oncogenes for the treatment of cancer, the discovery of Gleevec provides a proof of the principle that rational molecular therapeutics can work. Moreover, experimental evidence from many groups shows in transgenic mouse models that oncogene-induced cancers may generally be reversible on oncogene inactivation (5–8). Although cancers in mice models may be less genomically complex than their human equivalents, these studies illustrate that under some circumstances, cancer is reversible. Thus, the challenge is to understand these circumstances and then identify drugs that can safely and effectively be used to inactivate oncogenes for the treatment of cancer.

In this issue of Clinical Cancer Research, Yaari et al. have shown an important principle in the development of therapies for the treatment of cancer. Rather than directly target an oncogene of interest, disrupting an essential upstream regulator may more effectively inhibit the oncogene signaling pathways to reverse cancer. These results illustrate how an understanding of the molecular pathways that regulate activation of an oncogene may be used to identify a potential molecular target.

MYC and Human Cancer

The MYC family of proto-oncogenes encodes transcription factors that play a pivotal role in regulating cellular proliferation, cellular growth, differentiation, angiogenesis, adhesion, and apoptosis (5, 9–11). Each MYC family member—c-MYC, L-MYC, and N-MYC—has been shown to be associated with specific types of human cancer. Thus, c-MYC translocation in a lymphoma is almost pathognomonic for Burkitt’s lymphoma. N-MYC amplification is likewise frequently associated with neuroblastoma, and L-MYC amplification is associated with small cell lung carcinoma (12). MYC is overexpressed in almost half of human cancers, most commonly through epigenetic mechanisms. A critical feature of the regulation of the MYC protein is that it must be phosphorylated to become activated and stabilized. Phosphorylation of Ser62 stabilizes and extends the half-life of MYC whereas Thr58 phosphorylation targets the transcription factor for ubiquitin-mediated degradation. The phosphorylation of MYC is regulated through other oncogenes including RAS, AKT, and ERK (13). These observations suggest that one way to target MYC for the treatment of cancer would be to target the inactivation of the gene products that regulate MYC phosphorylation. Indeed, recent results from many groups strongly suggest that the inactivation of the MYC oncogene may be effective in the treatment of human cancer. Several different strategies have been considered for the development of drugs that target MYC (6, 7, 14).

RAS and Cancer

The RAS family of oncogenes is also commonly mutated in human cancer. The family comprises a group of low-molecular weight GTP-binding proteins that are anchored to the cytoplasmic face of the plasma membrane: H-RAS, K-RAS, and N-RAS. Of these three, only K-RAS is required for survival in mice (15). Under normal physiologic conditions, RAS proteins are responsible for transmitting signals from receptor tyrosine kinases, such as epidermal growth factor receptor, to downstream modulators of cell growth (16). About 20% of human tumors exhibit activating mutations in one or more of the RAS genes, and of these, K-RAS is most frequently found
to be mutated (about 85% of the total), followed by N-RAS (15%) and H-RAS (less than 1%; ref. 17). Under conditions of tumorigenesis, abnormal RAS signaling can lead to uncontrolled cell proliferation and resistance to apoptosis; furthermore, RAS effector pathways have been found to play a role in angiogenesis, expression of matrix metalloproteinases, and other processes that contribute to tumor invasion and metastasis (16). RAS and MYC are well known to cooperate to induce tumorigenesis. RAS signaling may affect MYC by two independent mechanisms. First, the phosphoinositide 3-kinase pathway works downstream of RAS to inhibit glycogen synthase-3, which is responsible for phosphorylating MYC at Thr58. As a result, MYC is not targeted for ubiquitin-mediated proteolysis. Second, RAS activates Raf-1, which in turn activates the extracellular signal-regulated kinase/mitogen-activated protein kinase signaling cascade, leading to phosphorylation of MYC at Ser62 and its subsequent protein stabilization (Fig. 1; ref. 16).

Because of the prevalence of RAS mutations in human cancers, much attention has been focused on the development of RAS inhibitors. One particular class of RAS inhibitors, farnesyltransferase inhibitors, has shown considerable promise in vitro and in vivo (16, 18–20). However, although exhibiting some antitumor activity in patients, their therapeutic potential has not been completely realized in clinical trials, most likely due to the particular manner in which farnesyltransferase inhibitors inhibit RAS activity (16, 21, 22). By preventing the addition of a farnesyl isoprenoid group to the COOH-terminal end of RAS proteins, farnesyltransferase inhibitors were thought to inhibit membrane association of RAS and thus inhibit RAS oncogenic activity. However, whereas farnesyltransferase inhibitors certainly antagonize the activity of H-Ras in cells in culture and in vivo, K-Ras and N-Ras can both be alternatively modified by geranylgeranyl transferase, resulting in the addition of a longer isoprenoid group (20 carbon atoms instead of 15) that allows for membrane association of the RAS proteins and presumable retention of oncogenic activity (16, 18, 23). The RAS inhibitor used by Yaari et al., however, represents a novel class of RAS inhibitors that resemble farnesylcysteine on the Ras protein. Farnesyl thiosalicylic acid, the most well studied of these compounds, disrupts the membrane association of all RAS proteins without producing cytotoxic effects in normal cells and has been shown to inhibit RAS transformation in vitro and in vivo (Fig. 2; refs. 24–26).

Targeting N-MYC-RAS with Farnesyl Thiosalicylic Acid to Treat Neuroblastoma

Neuroblastoma accounts for 7% to 10% of all pediatric neoplasms and as many as 50% of all malignancies diagnosed in infancy (27). Neuroblastomas generally comprise a spectrum of neuroblastic tumors that arise from primitive sympathetic ganglion cells and histologically appear as characteristic small, blue, round cell tumors (28). These tumors can manifest in a wide range of clinical behaviors, from spontaneous regression to aggressive metastasis leading to death (29). Several prognostic factors have been identified, most notably amplification of the N-MYC oncogene. The greater the number of copies of N-MYC, the more aggressive the disease and the poorer the outcome (30).

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**Fig. 1.** Ras signaling affects MYC via two independent pathways that act synergistically to stabilize MYC. First, acting through Raf-1 and extracellular signal-regulated kinase (mitogen-activated protein kinase), Ras phosphorylates MYC at Ser62, thereby extending the half life of MYC. Concurrently, Ras signals through phosphoinositide 3-kinase to block the activity of glycogen synthase-3, thereby preventing phosphorylation of MYC at Thr58 and preventing its proteolytic degradation.
Yaari et al. set out to explore the possibility of targeting not simply the associative genetic lesion (N-MYC amplification) but also the cooperation between oncoproteins. The idea that active RAS may also contribute to the disease progression of neuroblastoma has not yet been explored. Mutations in the RAS genes have rarely been found in neuroblastomas (31). Yaari et al. employed a novel inhibitor of the RAS protein, farnesyl thiosalicylic acid, to evaluate the importance of the N-MYC-RAS cooperation to the neoplastic phenotype of a neuroblastoma cell line. Administering farnesyl thiosalicylic acid to LAN-1 neuroblastoma cells results in significant decreases in levels of membrane-associated and active RAS proteins as well as in levels of downstream effectors of the RAS signaling cascade such as Raf-1, phospho-extracellular signal-regulated kinase, phosphoinositide 3-kinase, and phospho-AKT. Furthermore, the authors observe a dose-dependent inhibition of LAN-1 cell growth without cytotoxicity, and importantly, they also note a dramatic decrease in N-MYC levels by both farnesyl thiosalicylic acid and introduction of a dominant-negative RAS mutant. Hence, this study shows that N-MYC activity depends on RAS activation for stabilization in neuroblastoma cell lines and suggests that RAS inhibitors may prove effective in the treatment of advanced stage neuroblastoma and other MYC-induced neoplasia. This study provides an example of the principle that targeting upstream pathways may be an efficacious strategy for targeting MYC.

**Future Directions**

Experiments like those done by Yaari and colleagues are part of the emerging therapeutic paradigm of targeting critical signaling pathways required to sustain the activation of oncoproteins, rather than targeting the oncoproteins directly. There are several reasons to target multiple oncogenic pathways for cancer therapy. Such an approach may prevent tumor escape and relapse via activation of multiple pathways (32, 33). However, the disappointing experience with farnesyltransferase inhibitors in the clinic is a reminder that promising results in preclinical models do not necessarily translate to clinical utility. One difficulty has been in the development of a method in the evaluation of a drug on successful targeting of a protein. In this regard, the very recent development of proteomic methods for a more iterative analysis of cancer signaling pathways may provide just the platform needed to validate the efficacy of drugs when investigated in clinical trials (34, 35).

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**Fig. 2.** Two means for targeting RAS. Farnesyltransferase inhibitors (FTI) prevent the addition of an farnesyl isoprenoid group required for membrane association and thus RAS activity (a). However, N-RAS and K-RAS can circumvent this inhibition via alternative modification by geranylgeranylation transferase. By contrast, farnesyl thiosalicylic acid (FTS) disrupts the membrane association of all RAS proteins, and thus subsequent RAS signaling cascades, by displacement of RAS from the cell membrane (b).
Clinical Cancer Research

Getting at MYC through RAS
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