KRAS Mutations in Non–Small Cell Lung Cancer

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Some non–small cell lung cancers (NSCLCs) harbor a single specific mutated oncogene that is thought to be the primary genetic "driver" leading to cancer. The two most commonly mutated oncogenes in lung cancer encode for the epidermal growth factor receptor (*EGFR*) and *KRAS*. *EGFR* kinase domain mutations were only recently identified, but they have already been established in the clinic as valid predictors of increased sensitivity to EGFR kinase inhibitors (gefitinib and erlotinib). By contrast, even though *KRAS* mutations were identified in NSCLC tumors more than 20 years ago, we have only just begun to appreciate the clinical value of *KRAS* tumor status. Recent studies indicate that patients with mutant KRAS tumors fail to benefit from adjuvant chemotherapy, and their disease does not respond to EGFR inhibitors. There is a dire need for therapies specifically for patients with *KRAS* mutant NSCLC. In this review, we summarize the initial discovery of *RAS* mutations in NSCLC, describe work exploring associations with clinical factors and outcomes, and provide an overview of current approaches to targeting *KRAS* mutant NSCLC.

Keywords: non–small cell lung cancer; epidermal growth factor receptor; *KRAS*; mutations

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), gefitinib (Iressa) and erlotinib (Tarceva), induce dramatic responses in certain patients with non–small cell lung cancer (NSCLC). As such, the drugs have provided an unexpected tool to dissect clinically relevant molecular subsets of NSCLC. For example, using mutational profiling of tumor DNA from patients with known clinical outcomes to these drugs, we have demonstrated that somatic mutations in the tyrosine kinase domain of *EGFR* are associated with sensitivity to gefitinib and erlotinib (1), while mutations in *KRAS,* which encodes a GTPase downstream of EGFR, are associated with primary resistance (2). *EGFR* mutations are more commonly found in tumors from patients who never smoked cigarettes (1), while *KRAS* mutations are present in those with significant tobacco exposure (2). Moreover, in our analysis of 300 patients with tumors resected at Memorial Sloan-Kettering Cancer Center (MSKCC) and never treated with kinase inhibitors, *EGFR* and *KRAS* mutations were associated with distinct prognoses; patients whose tumors had *EGFR* mutations had a longer overall survival than those whose tumors had *KRAS* mutations (3). Thus, these two mutations define distinct populations of patients with NSCLC with different natural histories and responses to targeted therapy. Multiple reviews have been written about *EGFR* mutations in lung cancer (4–9). Below, we focus on the role of *KRAS* mutations in this disease.

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RAS GENES

RAS genes, like many oncogenes, were originally discovered through the study of cancer-causing retroviruses in animals. RAS-related investigations began in the early 1960s, when researchers observed that a preparation of a mouse leukemia virus, taken from a leukemic rat, induced sarcomas in rodents (Figure 1) (10). A similar type of retrovirus was identified in 1967, by serial passage of mouse leukemia viruses through rats (11). These two rat sarcoma (ras)-inducing retroviruses, named after their discoverers (Harvey and Kirsten, respectively), were later found to carry sequences derived from the rat genome (12). In 1982, multiple groups reported molecular cloning of transforming genes from human cancer cell lines. These genes turned out to be the human homologs of rat Harvey (*Ha-* or *H-*) ras and Kirsten (*Ki-* or *K-*) *ras* (13–15). Another *ras* family gene, neuroblastoma- or *N-ras*, was identified a year later (16, 17). Today, we know that the *RAS* genes encode a family of membrane-bound 21-kd guanosine triphosphate (GTP)-binding proteins that regulate cell growth, differentiation, and apoptosis by interacting with multiple effectors, including those in the MAPK (mitogen-activated protein kinase), STAT (signal transducer and activator of transcription), and PI3K (phosphoinositide 3-kinase) signaling cascades (18–20).

RAS AND NON–SMALL CELL LUNG CANCER

In 1982, molecular cloning of normal human *HRAS* and its oncogenic allele allowed investigators to establish that the functional differences between the two were caused by a single point mutation (21–23). *KRAS* and *NRAS* were found similarly to be activated by point mutations. Subsequently, a landmark study in 1984 demonstrated that a human lung cancer specimen contained an activating *KRAS* mutation that was not found in corresponding normal tissue (24). These data confirmed that observations found in human cell lines were indeed relevant and that somatic mutations occurred in human cancers. Shortly thereafter, investigators found that lung cancers frequently harbor somatic *KRAS* mutations (25).

Today, we know that RAS proteins acquire transforming potential when an amino acid at position 12, 13, or 61 is replaced as a result of a point mutation in the gene (26). These mutations lead to forms of RAS that have impaired GTPase activity, leading to constitutive activation of RAS signaling. *RAS* mutations are found in approximately one-third of all human malignancies (26). *KRAS* accounts for most of the *RAS* mutations found in the majority of human malignancies. Notably, *KRAS* accounts for 90% of *RAS* mutations in lung adenocarcinomas, and approximately 97% of *KRAS* mutations in NSCLC involve codons 12 or 13 (27). *KRAS* mutations are uncommon in lung squamous cell carcinomas (28, 29).

KRAS MUTATIONS AND CIGARETTE SMOKING IN NSCLC

Since *KRAS* mutations are common in NSCLC, and since cigarette smoking is a frequent cause of NSCLC, *KRAS* mutations have been widely hypothesized to be related to direct tobacco exposure. However, analyses attempting to associate

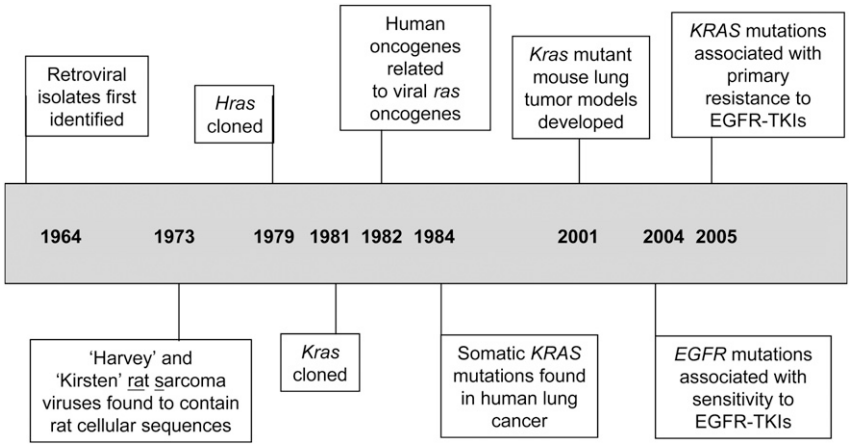

Figure 1. Timeline for observations about KRAS relevant to non–small cell lung cancer. EGFR-TKIs: epidermal growth factor receptor tyrosine kinase inhibitors.

smoking history with *KRAS* mutations have suffered from an absence of detailed patient smoking histories (i.e., intensity of smoking, duration of smoking), and most reports have studied only relatively small numbers of never-smokers with NSCLC (defined as those individuals who smoked < 100 cigarettes in a lifetime) (1). Moreover, despite high frequencies of *KRAS* mutations in colorectal cancer, colon cancer has not been clearly associated with smoking.

Recently, we evaluated the frequency of *KRAS* mutations in lung adenocarcinomas from nearly 500 patients, of whom 17% had never smoked cigarettes (30). We noted that *KRAS* mutations occurred in 22% of the overall population and in 15% of lung adenocarcinomas from never-smokers. *KRAS* transition mutations (G→A) were more common in patients who had never smoked cigarettes. In contrast, transversion mutations (G→T or G→C) were more common in former/current smokers. These data suggest that while some mutations in *KRAS* are associated with cigarette smoking, *KRAS* mutations do occur in never-smokers. Thus, unlike *EGFR* mutations, which occur more frequently in tumors from never-smokers (31), *KRAS* tumor status cannot be easily predicted on the basis of smoking history alone.

KRAS MUTATIONS AS A PROGNOSTIC FACTOR IN NSCLC

Early studies reported that in patients with resected NSCLC, those with *KRAS* mutations had a poor overall outcome, while other researchers noted no negative prognostic value to *KRAS* mutations. More recently, Mascaux and colleagues performed a meta-analysis of more than 53 studies, which evaluated *KRAS* mutations and outcomes in patients with NSCLC (32). They identified *KRAS* mutations as a negative prognostic factor with a hazard ratio (HR) for death of 1.40 (95% confidence interval [CI], 1.18–1.65). Among adenocarcinomas—the histology most likely to have *KRAS* mutations—the HR was 1.50 (95% CI, 1.26–1.80). Unfortunately, since all prognostic factors were not available for all studies, the authors were not able to perform a multivariate analysis including other prognostic variables such as stage, performance status, and weight loss. In addition, since the meta-analysis included only published studies, a publication bias (with studies not showing a prognostic significance for *KRAS* mutations going unpublished) is likely to make the hazard ratio artificially elevated. The optimal approach to determine the prognostic significance of *KRAS* mutations is to obtain *KRAS* mutation status prospectively as part of a clinical trial.

The first reported large trial that prospectively assessed *KRAS* mutations was conducted as part of E3590, a randomized trial in which patients with stage II–IIIA NSCLC were randomized to receive postoperative radiation therapy or radiation therapy and chemotherapy (33). Of the 488 patients who enrolled in E3590, tumors from 197 were available for *KRAS* mutational analysis, and mutations were identified in 24%. For patients on the chemotherapy arm of the study, the 70 patients who had wild-type *KRAS* had a median survival of 42 months, compared with 25 months for the 20 patients with *KRAS* mutations (risk ratio of wild type:mutant *KRAS*, 0.59; $P = 0.09$). In patients with good performance status, there was no prognostic significance for *KRAS* mutations (risk ratio, 1.08; $P = 0.08$, for wild-type versus mutant *KRAS*). Further, in multivariate analysis, *KRAS* mutation was not an independent prognostic factor, suggesting that *KRAS* mutation did not carry a distinct prognosis in this sample of patients with resected NSCLC.

KRAS MUTATIONS AS A PREDICTIVE MARKER OF THERAPY FOR NSCLC

Data are emerging that *KRAS* mutation status may assist in the prediction of clinical outcomes for patients receiving various treatments. One example exists in the adjuvant setting. Since only approximately 10% of patients who receive adjuvant chemotherapy for NSCLC derive benefit (34), an accurate predictive marker could decrease the frequency of administration of chemotherapy to patients who are unlikely to benefit. Recently, the National Cancer Institute of Canada reported a preplanned *KRAS* mutational analysis from a prospective, randomized trial allocating patients with resected stage IB–II NSCLC to receive adjuvant cisplatin/vinorelbine or observation (35, 36). In the whole population, patients who received chemotherapy had an improvement in overall survival, with an HR of 0.70 ($P = 0.03$). Among 450 tumors (available for 94% of patients enrolled), 117 had *KRAS* mutations. In *KRAS* wild-type patients, the HR for treatment with cisplatin and vinorelbine remained significant (HR, 0.69; $P = 0.03$). However, in those patients whose tumors had *KRAS* mutations, there was no difference in overall survival for patients treated with observation versus chemotherapy (HR, 0.95; $P = 0.87$). *KRAS* mutation was not a significant prognostic marker for survival in univariate or multivariate analyses. Taken together, these prospectively collected data suggest that there is no prognostic significance for *KRAS* mutations in this cohort of patients with early-stage NSCLC and that chemotherapy with cisplatin and vinorelbine is unlikely to benefit patients whose tumors have *KRAS* mutations.

TABLE 1. ANALYSES OF *KRAS* MUTATIONS AND EFFICACY OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS IN NON–SMALL CELL LUNG CANCER

Author	Drugs	Patients Tested for *KRAS* Mutations (*Total Number Mutant*)	Response Rate in *KRAS* Mutant
Pao (2)	Gefitinib/erlotinib	59 (9)	0%
Jackman (47)	Erlotinib	41 (6)	0%
Zhu (48)	Erlotinib	206 (30)	5%
Miller (49)	Erlotinib	80 (18)	0%
Massarelli (50)	Gefitinib/erlotinib	70 (16)	0%
Hirsch (51)	Gefitinib	138 (36)	1%
Hirsch (52)	Gefitinib	152 (12)	0%
Han (53)	Gefitinib	69 (9)	0%
Van Zandwijk (54)	Gefitinib	15 (3)	0%
Fujimoto (55)	Gefitinib	31 (7)	0%
Felip (56)	Erlotinib	39 (7)	0%

In patients with metastatic NSCLC, *KRAS* mutations have been investigated as negative predictors of benefit from erlotinib or gefitinib treatment. Since *KRAS* is a downstream effector of EGFR, the target of erlotinib and gefitinib, we hypothesized that inhibition of EGFR would be ineffective in controlling tumors with *KRAS* mutations. In 2005, we examined the tumor *KRAS* status in patients with NSCLC who had been treated with either drug as a single agent (2). Collectively, none of 21 patients whose disease responded radiographically had *KRAS* mutations, while 9 of 38 patients with refractory disease had *KRAS* mutations ($P = 0.02$). Multiple other groups have reported similar findings (Table 1). Remarkably, treatment of patients with colorectal cancer with cetuximab and panitumumab (antibodies directed against EGFR) has been demonstrated to be ineffective in patients whose colorectal tumors have *KRAS* mutations (37–40). These data suggested that anti-EGFR therapy in general may be ineffective against *KRAS* mutant tumors across multiple cancer types.

The role of *KRAS* mutations as a predictor of response for patients with stage IV NSCLC treated with chemotherapy alone is poorly understood. However, intriguing data were reported from a molecular analysis of tumors from patients enrolled in the phase III TRIBUTE trial (chemotherapy plus placebo versus chemotherapy plus erlotinib for previously untreated patients with NSCLC) (41). Of the 274 tumors available (from a total of 1,079) for *KRAS* mutational analysis, 55 had *KRAS* mutations. In patients with *KRAS* mutant tumors, the response rate for erlotinib plus carboplatin and paclitaxel (8%) was lower than that for patients who received the chemotherapy doublet alone (23%), with an overall survival HR of 2.1 (95% CI, 1.1–3.8). These data suggest that treatment with erlotinib not only does not improve the overall survival for patients with *KRAS* mutations treated with carboplatin and paclitaxel, but may also in fact decrease the efficacy of chemotherapy in that population. Of note, the response rate for patients treated with carboplatin and paclitaxel did not differ significantly by *KRAS* mutation status (26% versus 23%). In plots of overall survival and progression-free survival, stratified by treatment and by *EGFR* and *KRAS* mutation status, patients with *KRAS* mutations who were treated with erlotinib along with chemotherapy had the shortest overall survival.

CURRENT THERAPEUTIC APPROACHES BASED ON INHIBITION OF RAS-MEDIATED SIGNALING

Because RAS is commonly altered in human malignancies, it has attracted considerable attention as a target for anticancer therapy (42–45). Thus far, however, the quest for therapeutic inhibitors of RAS has fallen short of expectations. An obstacle to development of specific RAS inhibitors is that mutated, RAS proteins (which have gained constitutive activity) have lost their normal enzymatic function. Such loss-of-function mutated enzymes are much more difficult to inhibit than gain-of-function activated enzymes, such as BCR-ABL (the target of imatinib) and mutant EGFR (the target of erlotinib and gefitinib).

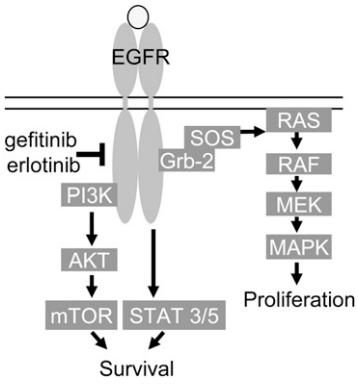

Figure 2. Simplified schematic of epidermal growth factor receptor (EGFR) and KRAS signaling pathways. The GTPase, KRAS, is downstream of EGFR, which signals through the PI3K/AKT/mTOR and STAT pathways involved in cell survival, and the RAS/RAF/MEK/MAPK pathway involved in cell proliferation. Gefitinib and erlotinib block the kinase activity of EGFR.

Currently, no direct RAS inhibitors have proven clinically effective, but development of agents to inhibit RAS has been pursued widely. Therapeutic approaches fall into three major classes. The first class attempts to inhibit RAS protein synthesis (e.g., *RAS* antisense oligonucleotides). This type of agent has been difficult to develop due to drug delivery issues. The second class alters RAS membrane localization. This includes agents such as farnesyl protein transferase inhibitors, which prevent necessary post-translational modification, or farnesylthiosalicylic acid (salirasib), which mimics the carboxy terminal amino acid of RAS and dislodges activated RAS from the membrane. Despite a large number of clinical trials, farnesyl transferase inhibitors have not demonstrated efficacy against tumors containing activated KRAS. The failure of this class of drugs is likely a result of alternative cellular pathways for post-translational modification of KRAS (as opposed to HRAS) (46). Moreover, FTIs may act through targets other than RAS (43). Clinical trials with salarisib are ongoing in both NSCLC and pancreatic cancer. The third class of anti-RAS agents bypasses RAS and inhibits effector molecules downstream of the mutant GTPase (e.g., RAF-, MEK-, PI3K-, and AKT-inhibitors) (Figure 2). These types of agents may hold promise, but testing in human patients is still in early phases.

CONCLUSIONS

Although *KRAS* mutations were identified in NSCLC more than 20 years ago, they have only recently come to be appreciated as biomarkers of response to specific anti-cancer agents. Emerging data suggest that *KRAS* mutations are negative predictors of benefit from both adjuvant chemotherapy and anti-EGFR–directed therapies. Further efforts to develop therapies for patients with KRAS mutant NSCLCs are urgently needed. As one step toward this future goal, we at MSKCC have been routinely genotyping patients' tumors for *KRAS* mutations and are conducting clinical trials specifically for patients with KRAS mutant tumors in both the adjuvant and metastatic settings.

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