Molecular Pathways: Targeting NRAS in Melanoma and Acute Myelogenous Leukemia

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Abstract

Successful targeting of specific oncogenic “driver” mutations with small-molecule inhibitors has represented a major advance in cancer therapeutics over the past 10 to 15 years. The most common activating oncogene in human malignancy, RAS (rat sarcoma), has proved to be an elusive target. Activating mutations in RAS induce mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase-AKT pathway signaling and drive malignant progression in up to 30% of cancers. Oncogenic NRAS mutations occur in several cancer types, notably melanoma, acute myelogenous leukemia (AML), and less commonly, colon adenocarcinoma, thyroid carcinoma, and other hematologic malignancies. Although NRAS-mutant tumors have been recalcitrant to targeted therapeutic strategies historically, newer agents targeting MAP/ERK kinase 1 (MEK1)/2 have recently shown signs of clinical efficacy as monotherapy. Combination strategies of MEK inhibitors with other targeted agents have strong preclinical support and are being evaluated in clinical trials. This review discusses the recent preclinical and clinical studies about the role of NRAS in cancer, with a focus on melanoma and AML.

Background

Wild-type NRAS

Three RAS (rat sarcoma) family members are frequently mutated across the spectrum of malignancy: NRAS (neuroblastoma RAS), KRAS (Kirsten RAS), and HRAS (Harvey RAS). Ras proteins comprise a family of low-molecular-weight GTPases. Wild-type RAS serves a critical role in cellular proliferation; KRAS knockout mice are characterized by embryonic lethality due to liver insufficiency and anemia (1). NRAS and HRAS seem to be more dispensable; knockout mice have mildly immune-deficient and normal phenotypes, respectively, suggesting that expression of these genes is less ubiquitous (2).

RAS proteins function as a conduit for signals received from receptor tyrosine kinases (RTK) on the cell surface through downstream cell signaling partners to nuclear transcription factors regulating cell growth and cell-cycling proteins. Under physiologic conditions, RAS activation is initiated by binding of an upstream RTK to its ligand (see Fig. 1). This interaction induces RTK autophosphorylation, dimerization, and activation. Adaptor molecule recruitment is triggered (such as growth factor receptor–bound protein 2, grb2), which subsequently recruits one of a family of guanine nucleotide exchange factors (GEF). These GEFs catalyze the rate-limiting step of RAS activation, the exchange of a GDP for a GTP, and include son of sevenless homolog 1 (SOS1), SOS2, and Ras protein-specific guanine nucleotide–releasing factor (3). A number of GTPase-activating proteins (GAP), notably including neurofibromin 1 (NF1), function as RAS suppressors and oppose this activation step. Once activated, RAS signals through a variety of downstream targets, most notably the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)–AKT, and Ral–guanine nucleotide dissociation stimulator (GDS) pathways, to induce cell growth and proliferation. Many other RAS targets have also been described, including AF-6, Ras, and Rab interactor 1 (RIN1), and phospholipase C, but their role in normal and aberrant signaling is unknown.

Mutant NRAS

Oncogenic activation of RAS has been described in 20% to 30% of human cancers (4–8). RAS is named for a retrovirus that induced murine sarcomas that were later found to haveactivating RAS mutations (9). RAS alterations were initially identified in 1983 on chromosome 1 in neuroblastoma, closely following the identification of KRAS and HRAS (10, 11). Constitutive activation in the setting of malignancy is caused by a single-point mutation, almost exclusively occurring in codons 12, 13, and 61. Mutations in codon 61 induce RAS activation by disrupting GTPase activity, and thereby locking RAS into its active conformation. Codon 12 and 13 mutations produce the same overall effect by decreasing sensitivity to the GAPs (12). Oncogenic mutations in codons 12 and 13 predominate in KRAS and HRAS across the spectrum of...
malignancies. NRAS mutations occur much more often in codon 61 in both melanoma and acute myelogenous leukemia (AML) and most frequently involve an arginine-for-glutamine substitution (Q61R; ref. 13). Notably, inactivating mutations or deletions in NF1, a GAP, dysregulate NRAS and induces similar pathway activation as mutant NRAS.

NRAS mutations are present in the majority of congenital melanocytic nevi but occur only rarely in other benign melanocytic nevi. In contrast, mutations in BRAF are identified in the large majority of benign nevi (14, 15). This suggests that NRAS mutations are an early, critical oncogenic event in melanomagenesis but are not sufficient to induce invasive melanoma without cooperating genetic events [such as cyclin-dependent kinase (CDK)/retinoblastoma (Rb) pathway alterations or loss of p53; ref. 16]. The role of NRAS in oncogenic progression in AML is less well understood. An analysis of samples from patients with myelodysplastic syndromes (MDS) and AML arising from MDS identified only a modest increase in the frequency of NRAS mutations in the secondary AML cohort compared with the MDS group (11% vs. 5.7%), suggesting that NRAS mutations may be an early event in MDS (17). In contrast, mutations in other genes frequently altered in AML (such as NPM1, FLT3, RUNX1, and MLL) were present much more often in the AML samples compared with MDS. NRAS in colon adenocarcinoma may play a different pathophysiologic role than KRAS. In a mouse model, KRAS G12D caused hyperproliferation and induced invasive adenocarcinoma in conjunction with adenomatous polyposis coli loss, whereas NRAS G12D conferred resistance to apoptosis but did not induce cellular proliferation (18).

Constitutively activated NRAS signals through several pathways to mediate oncogenic effects, notably the MAPK, PI3K–AKT, and Ral–GDS pathways (3). Under physiologic conditions in normal melanocytes, wild-type-activated NRAS signals through dimers of BRAF rather than CRAF. In NRAS-mutant melanoma, extracellular signal–regulated kinase (ERK)–mediated feedback inactivates BRAF, RAF isoform switching occurs, and mutant NRAS mediates downstream signaling through CRAF (19). NRAS-induced MAPK signaling leads to cyclin D1 expression and cell-cycle dysregulation and promotion of prosurvival pathways (20, 21). Although the effects of mutant NRAS on PI3K–AKT and Ral–GDS signaling are less well characterized, there is evidence that these pathways have nonredundant functions in oncogenic transformation (22). PI3K, AKT3, or PTEN alterations rarely cooccur with NRAS mutations, suggesting that activated NRAS is sufficient to promote activation of this pathway (6). Oncogenic RAS also seems to promote metastases, immune evasion, metabolic reprogramming, and microenvironment remodeling (3, 13). Recent studies have shown NRAS-mutant melanomas to display a greater dependency upon the expression of the prosurvival protein Mcl-1 than do their BRAF-mutant counterparts (23).

**Clinical implications of NRAS mutations**

NRAS mutations are present in 15% to 20% of melanomas, 10% of AMLs, 1% to 2% of colon cancers, and 8% to 10% of thyroid cancers (13). NRAS mutations are also present in a variety of other hematologic malignancies,
including acute lymphocytic leukemia (11%), multiple myeloma (18%), MDS (5%), and chronic myelomonocytic leukemia (19%; refs. 24–26). In melanoma, NRAS confers distinct prognostic and histopathologic characteristics when compared with other genetic subtypes. NRAS-mutant melanomas has been associated with poor prognosis compared with BRAF-mutant and NRAS/BRAF wild-type melanomas. Furthermore, compared with BRAF-mutant melanomas, primary tumours are thicker, with more mitoses, but are less often ulcerated. In childhood AML, activating NRAS mutations commonly cooccur with NPM1 mutations and occur frequently in the favorable-risk population (27). No association with cytogenetic alterations or clinical outcomes has been identified. In colon adenocarcinoma, NRAS mutations do not seem to confer specific clinical features, although most series have assessed <10 NRAS-mutant colon cancers (28).

Currently, it is unclear whether all activating NRAS mutations induce a common oncogenic phenotype or whether particular amino acid substitutions in NRAS confer distinct clinical and prognostic features. Two small studies have suggested that KRA2G12V mutations conferred a worse prognosis compared with KRA2G12D in both lung and colon adenocarcinomas (29, 30). However, in hematologic malignancies (i.e., childhood acute lymphocytic leukemia), specific mutations in NRAS did not seem to induce differing clinical or prognostic features (31). In a retrospective study of colon adenocarcinoma, mutations in NRAS (any codon) or KRAS (codons 61, 117, and 146) seemed to confer resistance to the anti–epidermal growth factor receptor monoclonal antibody panitumumab in a similar fashion when compared with colon adenocarcinoma with KRAS codon 12 or 13 mutation (32).

Acquired mutations in NRAS (G12D/R, G13R, and Q61K/R/L) and KRAS (G12C, G12R, and Q61H) have emerged as resistance mechanisms to BRAF inhibitors. In one study, resistance to dabrafenib and vemurafenib occurred in 18% and 7% of patients with progressing BRAF-mutant melanoma, whereas another population demonstrated 18% of resistant tumors with NRAS mutations but none with KRAS mutations (33, 34). With so few tumors analyzed, it remains to be determined whether the combination of BRAF and MAP/ERK kinase (MEK) inhibitors will suppress the development of NRAS-mutant, BRAF inhibitor–resistant tumors.

Clinical–Translational Advances

Direct RAS targeting

Attempts to directly target NRAS with small-molecule inhibitors have been largely unsuccessful. The major class of directed RAS-targeted therapeutics investigated has been farnesyltransferase inhibitors (FTI). Farnesylation of a cysteine residue on the RAS oncoprotein before its insertion into the cell membrane is the primary translational modification essential for the transforming activity of RAS (35). Although preclinical activity was observed in RAS-mutant cell lines and animal models, clinical activity with this class of agents has been disappointing (36–38). In an unselected melanoma population, no responses were observed in 14 patients, despite potent inhibition of phosphorylated ERK (39). A single phase II trial in AML did demonstrate activity with FTI therapy (tipifarnib), with occasional complete responses; the mutational status of those responding patients was not reported (40). Off-target side effects were observed in these trials as numerous additional proteins crucial for cellular function were also likely inhibited.

RNA interference (RNAi) may be another potential approach to directly target NRAS. Rather than targeting the mutated protein, RNAi involves antisense oligonucleotides/small interfering RNAs (siRNA) that interfere with mRNA, inhibiting production of the oncoprotein (41). RNAi is a useful technique in preclinical models to thoroughly inhibit gene activity across a spectrum of mutations but has remained a challenge to incorporate clinically from a drug-delivery standpoint (42). These molecules lack stability in the circulation and require molecular modification for delivery of these nucleotides to tumors. A recent phase I study provided evidence that this approach may be feasible in clinical practice (43). This approach used nanoparticles packaged with siRNA designed to reduce the expression of ribonucleotide reductase M2 (RRM2) and demonstrated effective nanoparticle delivery to melanoma cells and decreased expression of RRM2. Despite the incredible promise of this technique, some technical hurdles remain before clinical development.

MEK inhibition

Because direct targeting of GTPases has proved difficult, many efforts have shifted to inhibiting downstream mediators of NRAS. In BRAF-mutant melanoma, inhibition of MEK is an effective therapeutic strategy that has improved overall survival (44). Thus far, MEK inhibitors also seem to be the most active class of agents against NRAS-mutant melanoma (44). Preclinical models demonstrated activity of MEK inhibition in NRAS-mutant melanoma (45). Despite strong mechanistic and preclinical rationale for using MEK inhibitors in RAS-mutated malignancies, their activity has been modest at best, with the exception of a single phase II trial (discussed below). Structural and functional analyses have identified a possible explanation for the differential sensitivity for BRAF- and RAS-mutant malignancies. An allosteric MEK inhibitor in development (cibemetinib; GDC-0973) that has demonstrated activity in BRAF-mutant melanoma potently inhibits phosphorylated MEK, which seems to be required to block MAPK signaling in BRAF-driven malignancies (46). Conversely, two MEK inhibitors earlier in the development process with more preclinical activity in KRAS-mutant cancers (GDC-0623 and G-573) induce a hydrogen bond interaction with the S212 codon of MEK, which inhibits phosphorylation by wild-type RAF. Other novel MEK inhibitors such as CH5126766 (RO5126766) function similarly and block the phosphorylation of MEK by reactivated CRAF, thereby inhibiting ERK signaling (47). Therefore, RAS-specific and
RAF-specific MEK inhibitors may be distinguished for future development.

Binimetinib (MEK162), a selective MEK1/2 inhibitor, seems to be the most active small-molecule inhibitor for NRAS-mutant melanoma currently in development. A phase II trial assessed this compound in both BRAF- and NRAS-mutant melanomas; objective responses (confirmed and unconfirmed) were observed in 20% of patients in the NRAS group, and an additional 43% had stable disease as their best response (48). The progression-free survival in this study was similar for both the NRAS-mutant cohort (3.7 months) and the BRAF-mutant group (3.6 months). Currently, an NRAS-mutant–specific phase III trial (NRAS melanoma and MEK inhibitor, NEMO) comparing binimetinib with dacarbazine is ongoing (NCT01763164). Other MEK inhibitors have been less well studied but may also have some activity in NRAS-mutant melanoma. Although no responses have been observed with selumetinib, trametinib induced temporary stable disease in 2 of 7 treated patients in a phase I study (49, 50). ROS126766 demonstrated a single objective response in a patient with NRAS-mutant melanoma in a phase I trial (51). Because single-agent MEK inhibitor therapies may have some activity and are generally well tolerated, there is great enthusiasm for clinical trials assessing MEK inhibitors as a component of rationally chosen combination regimens.

Fewer preclinical or clinical studies have been conducted with MEK inhibitors in hematologic malignancies. Nevertheless, a preclinical rationale exists to target MAPK signaling in NRAS- or NF1–mutated leukemia (52, 53). In AML, a phase II trial of selumetinib was conducted, with 7% of patients harboring NRAS mutations. None of these patients had an objective response, and modest activity was observed in the overall cohort (54). A genotype-unselected trial of trametinib in AML has recently completed accrual, although results have not yet been reported. In addition, a trial combining idarubicin, cytarabine, and binimetinib is planned for relapsed AML (NCT02049801).

Other inhibitors of MAPK signaling

Several other targeted agents are rational for use in NRAS-mutant melanoma on the basis of preclinical studies. Although the currently available RAF inhibitors induce paradoxical hyperactivation of ERK signaling in BRAF wild-type cells that promotes cancer growth, a new class of inhibitors that does not cause this phenomenon has been developed. One of these novel agents (PLX7904) demonstrated activity in vemurafenib-resistant cell lines that harbored a secondary NRAS Q61K mutation (55). SCH772984, an inhibitor of ERK1/2, the final common signaling component in the MAPK pathway, showed activity in xenograft models of BRAF- and NRAS-mutant melanomas (56). This agent has not entered clinical development, but other ERK inhibitors are in early-phase trials.

Combination therapy

Because NRAS activates multiple cell signaling pathways, single-agent MEK inhibition is likely insufficient to induce apoptosis and restrain tumor growth in most tumors. NRAS promotes both the MAPK and PI3K–AKT pathways; therefore, one obvious approach would be combining MEK inhibitors with agents blocking the PI3K–AKT pathway. A summary of NRAS-targeted therapies is presented in Fig. 2. Preclinically, there is significant rationale for dual pathway inhibition; additive activity was observed in NRAS-mutant cell lines with inhibition of both MAPK and PI3K–AKT signaling (57, 58). Currently, no clinical trials are recruiting that are restricted to RAS-mutant tumors, but several early-phase studies for advanced cancers are under way. The regimens under study include combination of trametinib and an AKT inhibitor (uprosertib; GSK2141795) in BRAF wild-type melanoma (NCT01941927) and in AML (NCT01907815). Binimetinib and several different PI3K/AKT pathway inhibitors are also being evaluated in early-stage trials (NCT01363232, NCT01337765, and NCT01449058).

Recent preclinical observations have also generated a great deal of interest in combining MEK inhibitors with CDK4/6 inhibitors. Full review of the CDK4/6 pathway is outside the scope of this review, but this pathway is briefly discussed here and reviewed elsewhere (59). The CDKN2A gene transcription product, p16INK4A, inhibits CDK4 and CDK6. CDK4/6 are serine/threonine kinases that phosphorylate Rb1, diminishing its ability to regulate the cell cycle. CDKN2A loss or CDK4/6–activating mutations/amplifications, therefore, inhibit Rb1 function and, thus, promote cell-cycle progression.

MAPK signaling and the cell-cycle regulatory pathways are dysregulated in nearly all melanomas, suggesting that cotargeting these pathways may be an attractive treatment strategy (6). In an inducible mouse model with an NRAS Q61K mutation on the background of CDKN2A loss, MEK inhibition with trametinib induced apoptosis but did not induce cell-cycle arrest or tumor regression. In contrast, extinction of NRAS by RNAi induced major tumor regression (60). When these molecular phenotypes were compared, CDK4 was identified as key to this differential effect. Subsequently, treatment with MEK and CDK4/6 inhibitors (trametinib and palbociclib, respectively) induced tumor regression, apoptosis, and cell-cycle arrest. On the basis of these preclinical results, a clinical trial assessing MEK and CDK4/6 inhibition with binimetinib and LEE011 (CDK4/6 inhibitor) in NRAS-mutant melanoma is now enrolling patients (NCT01781572). An additional phase I trial is being planned that combines trametinib and palbociclib (NCT02065063). If these agents prove effective, further trials will be needed to determine whether this combination is effective in other RAS-mutant malignancies or whether the effect is specific to melanoma.

Other combinations have demonstrated clinical or preclinical efficacy in NRAS-mutant melanoma. Sorafenib and tivantinib, a MET tyrosine kinase receptor inhibitor, were used in 8 patients with NRAS-mutant melanoma, with 2 patients achieving a complete or partial response, and 2 additional patients experiencing best responses of stable disease (61). Preclinical data also suggest that combining
inhibitors of MEK and WNT signaling, as well as AKT/NF-κB inhibition, may have value in NRAS-mutant melanoma (62, 63). Targeting the molecular chaperone heat shock protein 90 (HSP90) is one strategy that allows the simultaneous suppression of multiple downstream targets of RAS signaling. In a recent preclinical study, the HSP90 inhibitor XL888 was noted to have promising antitumor activity in a panel of NRAS-mutant melanoma cell lines, in part through suppression of CDK4, AKT, and WEE1 expression (23).

Immune-based therapy

The immune-based therapies [including high-dose interleukin 2, (IL-2), ipilimumab, and novel agents targeting the programmed cell death 1/ligand (PD1/PDL1) axis] are currently used in melanoma irrespective of genotype. Recent studies have suggested that immune therapy may confer increased benefit to the NRAS-mutant cohort. In a retrospective study, patients with NRAS-mutant melanoma had greater response rates to high-dose IL-2 compared with other genetic subgroups (64). In addition, we evaluated whether this observation extends to ipilimumab and anti–PD1/PDL1. Patients with NRAS-mutant melanoma had higher response rates to all immune therapy compared with those with NRAS/BRAF wild-type melanoma (32% vs. 18%), with an especially marked benefit with anti–PD1/PDL1 in a small sample (65). No mechanism has yet been identified, although NRAS-induced upregulation of melanoma lineage antigens or PDL1 may explain this finding. These studies will need to be confirmed prospectively with increased patient numbers. In AML and other hematologic malignancies, trials of anti–PD1/PDL1 are in early phases; no data yet exist for a genotype-specific effect in these settings.

Conclusions

Activating NRAS mutations play a critical role in oncogenesis in a large percentage of melanomas and hematologic malignancies. Mutated NRAS promotes oncogenesis through activated MAPK, PI3K, and Ral–GDS signaling and confers specific clinical and pathologic characteristics in melanoma; its phenotypic effects are less clear in other cancers. Targeting NRAS has been a challenge clinically. Single-agent MEK inhibitors are showing early signs of clinical efficacy. Strategies combining MEK inhibitors with agents targeting the CDK4/6 or PI3K–AKT pathway members are promising approaches to more effectively treat patients with melanoma, whereas NRAS targeting has lagged behind in the other malignancies. Rational combination strategies with a MEK inhibitor backbone may improve therapy for patients with RAS-mutated malignancies.
Disclosure of Potential Conflicts of Interest

J.A. Sosman is a consultant/advisory board member for Bristol-Myers Squibb and Genentech. No potential conflicts of interest were disclosed by the authors.

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References


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