Research Article
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Cancer Prevention Research

Lung-Cancer Chemoprevention by Induction of Synthetic Lethality in Mutant *KRAS* Premalignant Cells *In Vitro* and *In Vivo*

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Abstract

Lung cancer is the leading cause of cancer death in both men and women in the United States, with a low 5-year survival rate despite improved treatment strategies. These data underscore the great need for effective chemoprevention of this cancer. Mutations and activation of *KRAS* occur frequently in, and are thought to be a primary driver of the development of, non–small cell lung cancers (NSCLC) of the adenocarcinoma subtype. In this study, we developed a new approach for the chemoprevention of NSCLC involving specific targeting of apoptosis in mutant *KRAS* cells. This approach is based on a synthetic lethal interaction among TNF-related apoptosis-inducing ligand (TRAIL), the second mitochondria-derived activator of caspase Smac/DIABLO (Smac), and *KRAS*. Mutational activation of *KRAS* modulated the expression of TRAIL receptors by upregulating death receptors and downregulating decoy receptors. Furthermore, oncogenic *KRAS* repressed cellular FADD-like interleukin 1β–converting enzyme (FLICE)-like inhibitory protein (c-FLIP) expression through activation of Erk/mitogen-activated protein kinase (MAPK)-mediated activation of c-Myc. Smac overcame *KRAS*-induced cell-survival signaling by antagonizing X-linked inhibitor of apoptosis protein (XIAP). Therefore, the combination of TRAIL and a small molecule mimic of Smac induced apoptosis specifically in mutant *KRAS* cells without harming normal cells. We further showed that short-term, intermittent *in vivo* treatment with TRAIL and Smac mimic induced apoptosis in tumor cells and reduced tumor burden in a murine model of *KRAS*-induced lung cancer. These results reflect the potential benefit of a selective therapeutic approach for the chemoprevention of NSCLC. *Cancer Prev Res; 4(5); 666–73. ©2011 AACR.*

Introduction

Lung cancer has a 5-year U.S. survival rate of a discouraging 15% and continues to be the leading cause of cancer death in the United States and worldwide despite some improvement in survival over the past several decades (1, 2). A major reason for this dismal survival rate is that the majority of lung cancer patients are diagnosed at incurable late stages. Approximately 90% of lung cancer cases are related to tobacco smoking (3). Recent decreases in smoking have led to a downturn in lung cancer death rates in the United States, but smoking rates continue to increase worldwide. It is well known that the incidence of lung cancer decreases slowly after smoking cessation, ensuring that the lung cancer epidemic will continue for many years. Smoking cessation is clearly the most effective way to reduce lung cancer risk, but although reduced, the risk remains substantial in former smokers, who represent the majority of lung cancer diagnoses in the United States (4). Therefore, there is an urgent need to identify additional strategies to reduce the burden of lung cancer.

RAS genes encode a family of membrane-localized 21-kDa GTP-binding proteins that functions as a molecular switch linking receptor and nonreceptor tyrosine kinase activation to downstream cytoplasmic or nuclear events in regulating cell growth, differentiation, and apoptosis (5, 6). The RAS proteins interact with multiple effectors in the mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase (PI3K) signaling cascades (7). Found in approximately 30% of all human cancers, activating *RAS* mutations result in gene products of impaired guanosine triphosphatase (GTPase) activity that leads to constitutive activation of RAS signaling. *KRAS* accounts for most of the cancer-related *RAS* mutations, including in non–small cell lung cancers (NSCLC; refs. 8, 9). *KRAS* mutations also are present in individuals at risk (but without cancer) who have significant tobacco exposure and are detected in 25%

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to 40% of atypical adenomatous hyperplasia lesions, which are a potential precursor of adenocarcinoma (10). Therefore, KRAS mutations seem to be an early event in human NSCLC development and may be a good target for lung cancer prevention.

Years of research in the chemoprevention of lung cancer have been frustratingly unable to generate any effective agents in clinical trials (11). Certain agents, such as COX inhibitors, have shown some activity against lung cancer in animal models. They are, however, moderate in effect, require long-term continuous administration, and have safety concerns (11, 12) We proposed a new concept of therapy-like chemoprevention as a way to circumvent these common limitations of chemoprevention This approach is discussed in detail in Discussion.

TNF-related apoptosis-inducing ligand (TRAIL), or Apo2L, is a potent cell-death inducer (13, 14) and interacts with an unusually complex receptor system in humans comprising 2 death receptors and 3 decoy receptors (15). TRAIL binding to its death receptors 4 and 5 (DR4, DR5) leads to apoptosis by activating caspases 8 and 10 (16). The interaction of TRAIL with its decoy receptors 1 and 2 (DcR1, DcR2) and Osteoprotegerin (OPG), which lack functional cytoplasmic signaling domains, results in defective death signaling (17). Interest in TRAIL increased recently after TRAIL selectively induced apoptosis in a wide variety of transformed human tumor cell lines *in vitro* and as xenografts without affecting normal cells (18). TRAIL has not produced toxicity in cynomolgus monkeys and chimpanzees (19–21), and proapoptotic DR4 or DR5 agonists (e.g., the monoclonal antibodies mapatumumab and apomab) have produced little or no toxicity in phase-1 clinical trials (22, 23).

Another important protein in regulating apoptosis in cancer cells is the X-linked inhibitor of apoptosis protein (XIAP; ref. 24). Frequently overexpressed in cancer cells, XIAP negatively regulates apoptosis by directly inhibiting caspases 3 and 7 activity (25). XIAP activity is regulated by the second mitochondrial protein Smac/DIABLO (Smac). Release of Smac from mitochondria leads to its binding to XIAP and antagonizes the caspase–XIAP interaction, thereby promoting apoptosis (26, 27).

We recently reported results of a therapy-like approach for cancer chemoprevention involving the synthetic lethal interaction among TRAIL, retinyl acetate (RAc), and adenomatous polyposis coli (APC) signaling pathways (28). TRAIL plus RAc specifically induced apoptosis in APC-deficient cells through the activation of c-Myc and was effective chemoprevention of colorectal cancer in animal models (28). It has been shown that activation of *RAS* could lead to the activation of c-Myc (29). Therefore, TRAIL plus RAc has the potential to target the *RAS* signaling pathway in inducing apoptosis. However, *RAS* can also activate a prominent cell-survival pathway mediated by the activation of AKT, leading to inhibition of caspase activation by XIAP. We hypothesize that Smac would overcome these survival signals by antagonizing XIAP and thus facilitate apoptosis. This facilitation may provide a potent strategy for eliminating mutant *KRAS* premalignant cells and a therapeutic approach for preventing lung cancer. We report here our results with TRAIL plus a small-molecule Smac mimic in inducing apoptosis in *in vitro* and *in vivo* models of mutant *KRAS* lung premalignancy.

Materials and Methods

Plasmids, shRNAs, and reagents

The retroviral plasmid expressing a mutant *KRAS* ($KRAS^{V12}$) was reported earlier (30). Erk dominant-negative and ATK/PKB dominant-negative constructs were generously provided by Dr. Mien-Chie Hung of M.D. Anderson Cancer Center (31). The full-length cellular FADD-like interleukin 1β–converting enzyme (FLICE)-like inhibitory protein (c-FLIP) cDNA was generated by reverse transcriptase PCR of total RNA from Hela cells, as previously reported (28). The c-Myc-shRNA was described earlier (28). XIAP-shRNA was purchased from Openbiosystems. TRAIL was prepared according to published results (32). The small-molecule Smac mimic JP1584 was produced by Joyant Pharmaceuticals. U0126 and Wortmannin were purchased from CalBiochem.

Antibodies and Western blots

Anti–c-FLIP was obtained from ALEXIS Biochemicals. Anti–phospho-Erk, anti–phospho-AKT, anti–cleaved caspase 3 and anti-DR5 were purchased from Cell Signaling Technology. Anti–c-Myc was purchased from Millipore. Anti–β-actin and anti–α-tubulin were purchased from Sigma. Antibodies against DcR1 and DcR2 were purchased from Imgenex. Anti-DR4 was obtained from Upstate. Anti-XIAP was purchased from BD Transduction Laboratories. Anti–cleaved caspase 3 antibody was obtained from Western blotting was carried out as described previously (33).

Cell lines, infection, transfection, and drug treatment

Cells of the immortalized human bronchial epithelial (HBE) cell line HBE4-E6/E7 (HBE4) were purchased from ATCC. BW1799 cells were kindly provided by Dr. Reuben Lotan of M.D. Anderson Cancer Center and were maintained in Keratinocyte-SFM medium (Gibco). Retrovirus was generated by using the BOSC23 packaging cell line, and infection was carried out as reported previously (30). Transfections were carried out by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. For TRAIL and Smac mimic treatment, cells growing at the log phase were treated with Smac at a final concentration of 100 nmol/L for 4 hours. Then TRAIL was added to the media at a final concentration of 100 ng/mL. Cells were harvested after 24 hours of TRAIL treatment. Cells were treated with Wortmannin (200 nmol/L) and U0126 (10 μmol/L) for 30 minutes. Cell viability was determined by using Annexin V-FITC Apoptosis Detection Kit (Sigma) according to the manufacturer's instructions.

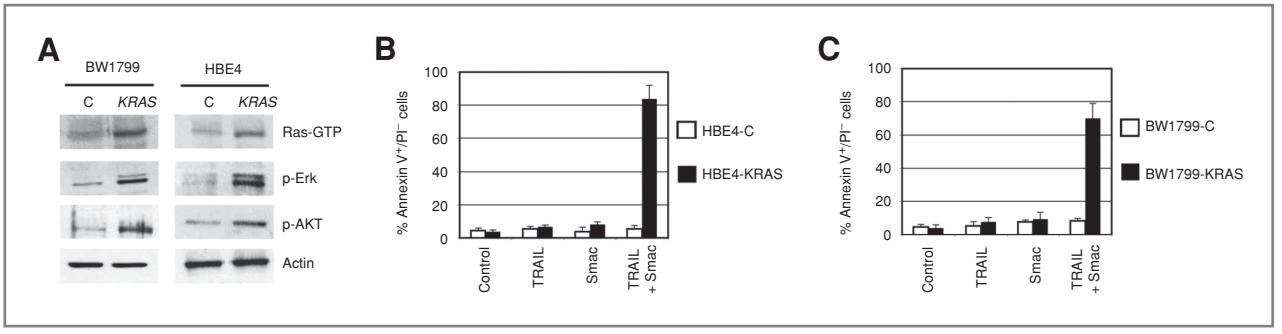

Figure 1. Oncogenic *KRAS* activates RAS downstream-signaling pathways and sensitizes HBE cells to TRAIL and Smac mimic. A, activation of Ras- GTP and induction of phosphorylation of Erk (p-ERK) and AKT (p-AKT) by *KRAS* in BW1799 and HBE4 cells. These cells were infected with either control (C) or retrovirus-expressing mutant *KRAS*. B, induction of apoptosis by TRAIL and Smac mimic. HBE4 cells and BW1799 cells expressing oncogenic *KRAS* were treated with TRAIL (100 ng/mL) or Smac mimic (100 nmol/L) or both or control (PBS) for 24 hours. Apoptotic cells (Annexin V^+, PI^-) were counted. The data represent results from 3 independent experiments. Averages and SD are shown.

Tumor studies in mice

Breeding colonies were established from LSL-K-rasG12D mice acquired from the Mouse Repository of the National Cancer Institute. AdenoCre virus was purchased from the Gene Therapy Core of Baylor College of Medicine. Mice were infected according to a previously reported protocol (34, 35). In brief, AdenoCre-calcium phosphate (AdCre-CaPi) precipitates were prepared by placing recombinant adenovirus in 1 mL of Eagle's minimal essential media containing 1.8 mmol/L Ca^{2+} and 0.86 mmol/L Pi. Then an aliquot of a 2 mol/L $CaCl_2$ solution was added to achieve a concentration of 4.5 mmol/L Ca^{2+}. The solution was mixed by vortex or gentle pipette tip aspiration, and the mixture was allowed to incubate for 20 to 30 minutes at room temperature. G12D mice were anesthetized with avertin at 6 weeks of age. AdCre-CaPi coprecipitates [5×10^8 pfu (plaque forming units)] were administered intranasally in two 62.5-μL instillations. The second instillation was administered when breathing rates had returned to normal. Six weeks after infection, the mice were injected intravenously with Smac mimic (3 mg/kg). Six hours later, TRAIL (3 mg/kg) was injected intraperitoneally. Injections were repeated 16 hours later. Consecutive injections of TRAIL and Smac mimic were given a total of 3 times. Injection of PBS was used as a control. For intermittent treatment, the mice were given 3 consecutive injections of TRAIL and Smac mimic (1.5 mg/kg) within a week, again 3 weeks later, and then again 3 weeks later, for a total of 3 treatment cycles. The mice were sacrificed 3 days after the last treatment, and their lungs were inflated with formalin and fixed in formalin overnight. Lung sections were analyzed by hematoxylin and eosin (H&E) and immunohistochemistry staining by using an anti–cleaved caspase 3 antibody, as reported previously (28).

Statistical analysis

We compared differences between groups via 1-way ANOVA. Values with a $P < 0.05$ were considered to be statistically significant.

Results

TRAIL and Smac mimic specifically induce apoptosis in *KRAS*-activated HBE cells

To test the effect of *RAS* activation on TRAIL plus Smac mimic–induced apoptosis, we introduced the activating mutant $KRAS^{V12}$ into immortalized normal HBE4 cells. Constitutive expression of mutant *KRAS* led to an increase in RAS-GTPase activity and activation of downstream signaling events, including phosphorylation of Erk and AKT in mutant *KRAS* HBE4 (Fig. 1A). Normal HBE4 cells were resistant to apoptosis induced by either TRAIL or Smac mimic alone or combined (Fig. 1B). Expression of mutant *KRAS*–sensitized cells to TRAIL plus Smac mimic–induced apoptosis (Fig. 1B). RAc had no effect on cell viability either alone or in combination with TRAIL and Smac mimic (data not show). Similar results occurred in BW1799 cells (Fig. 1A and C). These results suggest that activation of *KRAS* specifically sensitizes normal HBE cells to TRAIL and Smac mimic.

KRAS sensitizes cells to TRAIL and Smac mimic through the MAPK/Erk pathway

To explore the contribution of two RAS-regulated pathways (activation of MAPK/Erk and of AKT through PI3K) to TRAIL plus Smac mimic–induced apoptosis in mutant *KRAS* cells, we inhibited MAPK/Erk and AKT signaling with either dominant-negative mutants or pharmacologic inhibitors. Expression of dominant-negative mutants of Erks (Erk1 and Erk2) inhibited *KRAS*-mediated phosphorylation of Erks and TRAIL plus Smac mimic–induced apoptosis; inhibition of AKT phosphorylation by the expression of an AKT dominant-negative mutant did not affect TRAIL plus Smac mimic–mediated cell death in mutant *KRAS* cells (Fig. 2A and B). U0126, a MEK inhibitor upstream of Erk/MAPK, reduced Erk phosphorylation and blocked TRAIL plus Smac mimic–induced apoptosis in mutant *KRAS* cells, whereas the PI3K inhibitor Wortmannin blocked AKT phosphorylation but did not inhibit TRAIL plus Smac mimic–induced apoptosis (Fig. 2A and B). These results

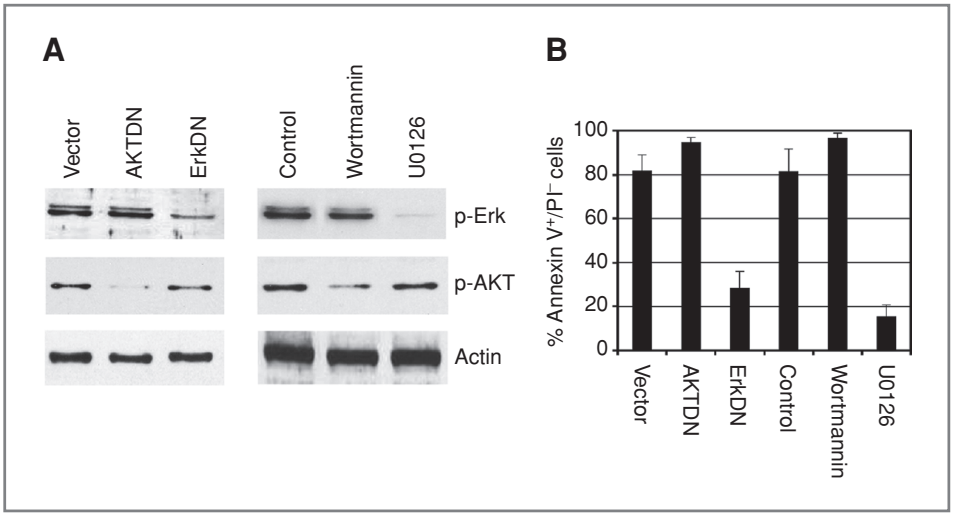

Figure 2. The Erk/MAPK pathway is required for *KRAS*-mediated sensitization to TRAIL and Smac. A, inhibition of *KRAS*-mediated activation of ERK and AKT. Phosphorylated Erk (p-ERK) and AKT (p-AKT) were assessed in HBE4-*KRAS* cells transfected with a dominant-negative AKT mutant (AKTDN) or both Erk1 and Erk2 dominant-negative mutants (ErkDN) or vector (control), or in these cells treated with the PI3K inhibitor Wortmannin (200 nmol/L) or the dual MEK1 and MEK2 inhibitor U0126 (10 μmol/L) or control (dimethyl sulfoxide). B, TRAIL- and Smac mimic–mediated apoptosis. The transfected or treated HBE4-*KRAS* cells were treated with TRAIL and Smac mimic for 24 hours. Apoptotic cells (Annexin V^+, PI^-) were counted. The data represent results from 3 independent experiments. Averages and SD are shown.

show that *KRAS* sensitized HBE cells to TRAIL and Smac mimic through the MAPK/Erk pathway.

c-Myc and c-FLIP are downstream mediators of *KRAS* in sensitizing cells to TRAIL and Smac mimic

To test our hypothesis that activation of *KRAS* induces the expression of c-Myc, which in turn inhibits the expression c-FLIP to sensitize cells to TRAIL and Smac mimic, we first analyzed the expression of c-Myc and c-FLIP in mutant *KRAS* cells. Expression of mutant *KRAS* resulted in enhanced levels of c-Myc protein and a corresponding decrease in c-FLIP protein expression (Fig. 3A). We next investigated the functional significance of c-Myc and c-FLIP. We first transfected *c-Myc*-shRNA into mutant *KRAS* cells to knock down c-Myc expression. Transfection of *c-Myc*-shRNA abolished *KRAS*-mediated induction of c-Myc expression and restored c-FLIP expression (Fig. 3B). More important, knockdown of c-Myc significantly inhibited TRAIL plus Smac mimic–induced apoptosis (Fig. 3D). Furthermore, restoring c-FLIP expression by transfecting a c-FLIP–expression plasmid-blocked TRAIL plus Smac mimic–induced cell death in mutant *KRAS* cells (Fig. 3C and D). These data support the ability of *KRAS* to sensitize normal HBE cells to TRAIL and Smac mimic through activating c-Myc and the subsequent repression of c-FLIP expression.

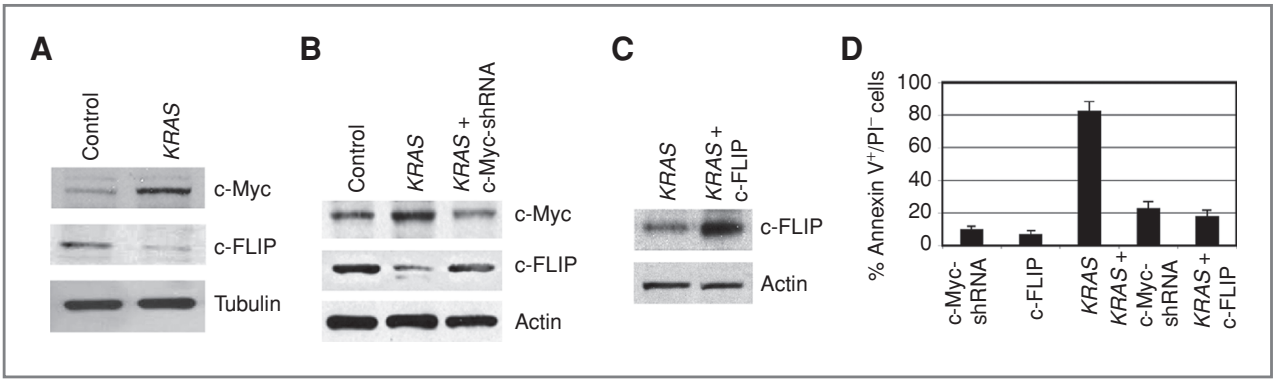

Figure 3. Involvement of c-Myc and c-FLIP in TRAIL plus Smac mimic–induced apoptosis in oncogenic *KRAS*–expressing cells. A, *KRAS*-mediated activation of c-Myc and repression of c-FLIP. HBE4 cells were infected with either control or *KRAS*-expressing retrovirus. B, effect of c-Myc knockdown. HBE4 cells were infected with either control or *KRAS*-expressing virus or were transfected with c-Myc-shRNA after *KRAS* infection. C, overexpression of c-FLIP. HBE4 cells infected with *KRAS* were transfected with either control vector or c-FLIP DNA. D, induction of apoptosis. HBE4 cells with various infection and transfection combinations were treated with TRAIL and Smac mimic. Apoptotic cells (Annexin V^+, PI^-) were counted. The data represent results from 3 independent experiments. Averages and SD are shown.

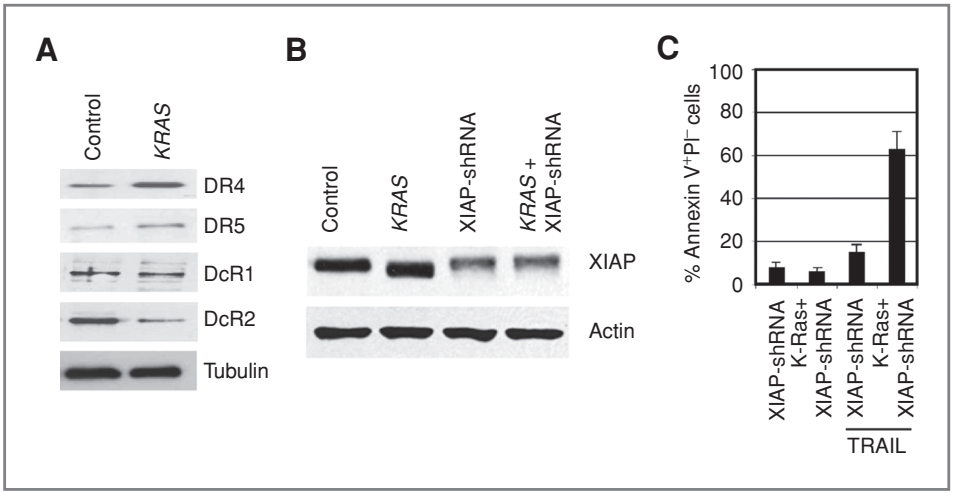

Figure 4. Modulation of TRAIL-receptor expression by *KRAS* and role of XIAP in TRAIL plus Smac mimic–induced apoptosis in oncogenic *KRAS*–expressing cells. A, effect of *KRAS* activation on the TRAIL receptors DR4, DR5, DcR1, and DcR2. HBE4 cells were infected with either control or *KRAS*-expressing retrovirus. B, effect of XIAP knockdown. HBE4 cells were infected with either control or *KRAS*-expressing virus or were transfected with XIAP-shRNA after *KRAS* infection. C, induction of apoptosis. HBE4 cells with various infection and transfection combinations were either treated with TRAIL or not treated. Apoptotic cells (Annexin V^+, PI^-) were counted. The data represent results from 3 independent experiments. Averages and SD are shown.

KRAS modulates the expression of TRAIL receptors and Smac mimic overcomes the antiapoptotic activity of XIAP to facilitate TRAIL-induced apoptosis

A surprising finding of our study was that RAc is not required for TRAIL plus Smac mimic–induced apoptosis in mutant *KRAS* cells, suggesting that *KRAS* may modulate the expression of TRAIL receptors, as RAc does (28). We tested this possibility by examining TRAIL receptor expression by Western blotting. Expression of mutant *KRAS* induced the expression of DR4 and DR5 (Fig. 4A). Mutant *KRAS* also inhibited the expression DcR2 significantly (Fig. 4A). These results indicate that expression of mutant *KRAS* enhances the DRs and represses the DcRs of TRAIL in facilitating TRAIL signaling. Mutant *KRAS* exerts an effect on TRAIL receptors similar to that of RAc and thus eliminated the need for adding RAc to TRAIL plus Smac mimic in inducing apoptosis in mutant *KRAS* cells.

We next searched for the target of Smac mimic in sensitizing mutant *KRAS* cells to TRAIL. We first analyzed XIAP expression in mutant *KRAS* cells by Western blotting. Although expression of XIAP was not affected significantly by the mutant *KRAS*, we detected significantly high levels of XIAP, suggesting that the antiapoptotic activity of XIAP needs to be inhibited to facilitate TRAIL signaling for apoptosis (Fig. 4B). To support this possibility, we used *XIAP*-shRNA to inhibit the expression of XIAP in mutant *KRAS* cells (Fig. 4B). Knockdown of XIAP sensitized mutant *KRAS* cells to TRAIL-induced apoptosis (Fig. 4C). As Smac mimic counters the activity of XIAP, these results support the role of Smac mimic in targeting the antiapoptotic activity of XIAP to facilitate TRAIL-induced apoptosis.

Short-term, intermittent TRAIL and Smac mimic treatments strongly inhibit lung tumor growth in mice

As TRAIL plus Smac mimic induced apoptosis in oncogenic *KRAS*–expressing cells *in vitro*, this combination has the potential to achieve chemoprevention through short-term and intermittent therapy. We tested this potential of TRAIL plus Smac mimic *in vivo* in a mouse model of mutant *KRAS* (*KRAS-G12D*)–driven lung adenocarcinoma. *KRAS-G12D* mice carry a conditional allele of oncogenic *KRAS-G12D* (LSL-*KRAS-G12D*) and closely mimic tumorigenesis initiated in human adults through somatic *KRAS* mutations (34, 35). LSL-*KRAS-G12D* contains a floxed transcriptional stop element, and infecting the lungs of these mice with AdenoCre virus, a recombinant adenovirus expressing the Cre recombinase, resulted in the expression of mutant *KRAS* and the development of epithelial hyperplasia of the bronchioles, adenomas, and eventually pulmonary adenocarcinomas (34). Six weeks after the induction of lung carcinogenesis in LSL-*KRAS-G12D* via AdenoCre-activated *KRAS-G12D* expression, the mice received 3 consecutive injections of TRAIL plus Smac mimic within 1 week. The mice were sacrificed 3 days after the treatments, and the lungs were examined for evidence of tumors by H&E staining. We used cleaved caspase 3 immunohistochemistry staining to test for apoptosis induction. TRAIL plus Smac mimic induced a significant level of apoptosis in lung tumor cells (Fig. 5A). There was no evidence of apoptosis in normal lung sections (Fig. 5A). Treated mice had a significantly decreased number of lung lesions (hyperplasias and adenomas; versus control mice; Fig. 5B). The most dramatic decrease was a 97% reduction in advanced lung lesions (adenomas; Fig. 5B). Therefore, short-term treatment

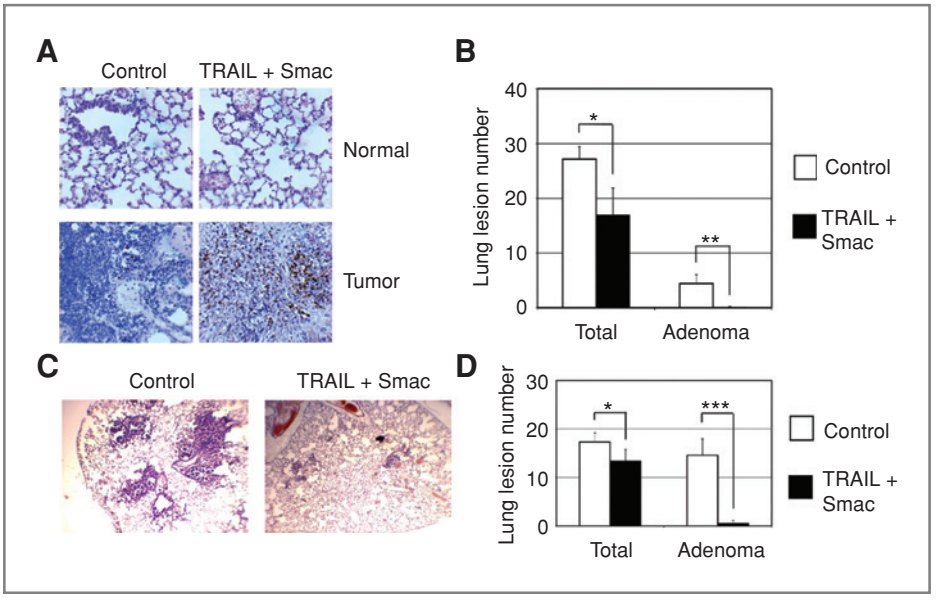

Figure 5. Effect of TRAIL and Smac on *KRAS*-induced lung tumors in mice. A, TRAIL and Smac mimic induce apoptosis in lung tumors of LSL-*KRAS*-G12D mice. LSL-*KRAS*-G12D mice were infected with AdenoCre and treated 6 weeks later with PBS (control; $n = 6$) or received 3 consecutive treatments with TRAIL (3 mg/kg) plus Smac mimic (3 mg/kg; $n = 6$) within 1 week. Mice without infection were used as normal controls. Three days after the last treatment, the lung sections were stained with an anti–cleaved caspase 3 antibody. Representative photomicrographs are shown. Caspase 3 staining was detected only in TRAIL/Samc-mimic treated tumor samples. B, TRAIL and Smac mimic treatment inhibits lung tumor growth. Lung sections were stained with H&E and lung lesions were counted. The data are derived from counting within serial sections in each mouse. Averages and SD are shown. *, $P < 0.05$; **, $P < 0.001$. C, intermittent treatment with TRAIL plus Smac mimic inhibits adenoma formation. LSL-*KRAS*-G12D mice were infected with AdenoCre. Six weeks later, the mice ($n = 6$ for each treatment group) were subjected to 3 rounds of intermittent treatment in 2 months with either PBS (control) or TRAIL (3 mg/kg) plus Smac mimic (1.5 mg/kg). Lung sections were stained with H&E; representative photomicrographs are shown. D, quantification of lung lesions. Serial sections were stained with H&E, and lung lesions were counted. The data are derived from counting within serial sections in each mouse. Averages and SD are shown. ***, $P < 0.0001$.

with TRAIL and Smac mimic inhibited *in vivo* lung tumor growth.

To test the effect of intermittent TRAIL and Smac mimic treatment, we treated the mice with 3 cycles of the 3-per-week consecutive treatments within 2 months. The mice were sacrificed 3 days after the last treatments, and the lungs were examined for evidence of tumors by H&E staining. At the time of analysis, most lesions in control mice were adenomas, whereas most lesions in treated mice were hyperplasias (Fig. 5C). A minor, but statistically significant, reduction was observed in the total number of lung lesions in the treatment group (versus controls; Fig. 5D). More important, the number of advanced lesions (adenomas) was greatly reduced in the treatment group (versus controls; Fig. 5D). The lower number of total lung lesions in Figure 5D than that in Figure 5B is likely due to some adenomas, which were more numerous in Figure 5D, comprising multiple hyperplastic lesions. These results support the ability of TRAIL plus Smac mimic to induce apoptosis in mutant *KRAS* cells *in vivo* and support the potential of this combination for therapy-like chemoprevention of human lung cancer.

Discussion

The concept of synthetic lethality originated in genetic experiments in yeast and fruit flies. In synthetically lethal gene pairs, a mutation of either gene alone is compatible with viability but mutation of both genes causes death (36–38). This concept has been applied in recent years in developing cancer-specific cytotoxic agents (39, 40). Synthetic lethality offers 2 advantages required for cancer treatment and chemoprevention. First, a synthetic lethality regimen has the ability to convert cytostatic targeting effects (which mainly stop cell division) to cell death, which is far less likely to result from directly targeting cancer-associated single mutations than from targeting the interactions between synthetic lethality mutations. Second, specific cancer-associated synthetic lethality interactions are associated with exquisite selectivity. This selectivity allows the killing of cancer cells without harm to normal cells that lack the cancer-specific mutation.

Our present results show for the first time a synthetic lethal interaction among TRAIL, Smac, and constitutive activation of *RAS* in premalignant bronchial epithelial cells (Fig. 6). This interaction relied on the following effects of *RAS* and Smac: *RAS* activates c-Myc and thus represses c-FLIP expression and modulates the expression of TRAIL receptors; and Smac overcomes the antiapoptotic effect of XIAP. These interactions allowed TRAIL plus Smac (Smac mimic) to kill *KRAS*-activated premalignant lung cells with great efficiency and specificity. As mutational activation of *RAS* occurs in approximately 30% of human cancers in general and of NSCLC in particular, targeting oncogenic

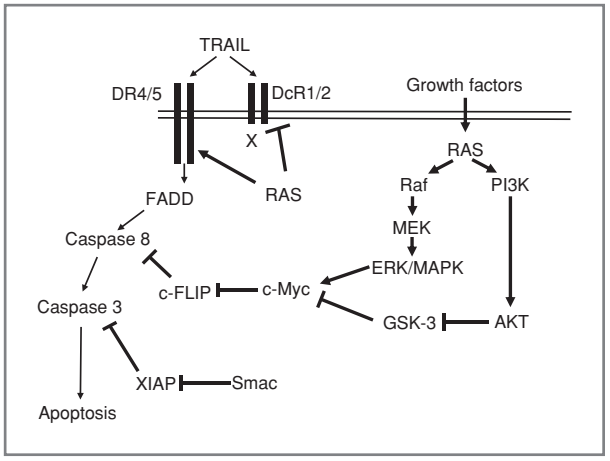

Figure 6. Synthetic lethal interaction among TRAIL, Smac, and RAS. Activation of RAS by growth factors or mutations leads to the activation of c-Myc through the activation of Raf, MEK, and Erk/MAPK and to the inhibition of GSK-3 through the activation of PI3K and AKT. Activation of c-Myc represses the expression of c-FLIP. RAS also induces the expression of DR4/5 and decreases the levels of DcR1/2. Smac inhibits the function of XIAP. Thus, TRAIL and Smac specifically cause apoptosis in cells with activated RAS.

RAS activation with the combination of TRAIL and Smac mimic is a potential new approach for the chemoprevention of NSCLC; this potential is supported by our present *in vivo* animal results.

Traditional chemoprevention efforts have focused on agents such as COX-2 inhibitors and dietary supplements, which modulate tumorigenesis without substantially eliminating premalignant cells. These agents have limited effects on tumor progression and often require continuous long-term application that raises the potential for unacceptable side effects. Producing synthetic lethal interactions by targeting tumor-specific gene mutations to eliminate premalignant cells through apoptosis is a new strategy for chemoprevention with the potential to overcome the 2 most pressing issues facing it today: effectiveness and toxicity. It is arguable that apoptosis induction is the most potent cellular anticancer mechanism and should be highly effective for chemoprevention. The safety of the approach in this study is achieved by the specificity that synthetic lethal interactions allow in targeting altered cells. Furthermore, therapy-like cancer prevention could be achieved by short-term and noncontinuous treatments to further reduce the potential side effects and costs often associated with long-term cancer chemoprevention. Therapy-like chemoprevention reflects a new direction for cancer prevention research.

We recently showed the efficiency and efficacy of this novel approach in colorectal carcinogenesis; TRAIL, RAc, and APC signaling pathways had a synthetic lethal interaction in inducing apoptosis in APC-deficient cells (28). This work further validates the applicability of this new approach to other oncogenic pathways and tumor types. We have provided preclinical evidence that short-term, intermittent TRAIL plus Smac mimic treatment is effective in inducing significant apoptosis and reducing premalignant lesions. These results support the use of TRAIL and Smac mimic for lung cancer chemoprevention in a therapy-like fashion. This combination potentially targets the signaling pathway leading to the activation of *RAS*, including the activation of growth factor signaling such as overexpression or mutational activation of the epidermal growth factor receptor. The activation of growth factor/*RAS* signaling pathways is frequently associated with various settings of human carcinogenesis. Therefore, TRAIL and Smac mimic could have chemopreventive potential against several human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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