miR-29b Mediates NF-κB Signaling in KRAS-Induced Non–Small Cell Lung Cancers

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Abstract

A global understanding of miRNA function in EGFR signaling pathways may provide insights into improving the management of KRAS-mutant lung cancers, which remain relatively recalcitrant to treatment. To identify miRNAs implicated in EGFR signaling, we transduced bronchial epithelial BEAS-2B cells with retroviral KRAS constructs and monitored miRNA expression patterns by microarray analysis. Through this approach, we defined miR-29b as an important target for upregulation by mutant KRAS in non–small cell lung cancers. Cell biologic analyses showed that pharmacologic inhibition of EGFR or MEK was sufficient to reduce levels of miR-29b, while PI3K inhibition had no effect. In KRAS-transduced BEAS-2B cells, introduction of anti-miR-29b constructs increased the sensitivity to apoptosis, arguing that miR-29b mediated apoptotic resistance conferred by mutant KRAS. Mechanistic investigations traced this effect to the ability of miR-29b to target TNFAIP3/A20, a negative regulator of NF-κB signaling.

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide with a mean 5-year survival rate of less than 15% (1). Non–small cell lung cancer (NSCLC) represents the majority of all lung cancers and is predominantly comprised of adenocarcinoma, which accounts for 40% of NSCLC. Other forms of NSCLCs are squamous cell carcinoma (SCC), large cell carcinoma, and further rare subtypes. Adenocarcinomas have their origin in the distant airways, whereas SCCs most often arise in the major bronchi (2). Treatment includes resection of the tumor and accompanying platinum-based chemotherapy. However, the majority of patients presenting with NSCLC cannot be cured due to advanced disease, and less than 30% of patients with metastatic NSCLC respond to platinum-based chemotherapy (3).

Activating mutations in the oncogenes KRAS (25%–40%) and EGFR (10%–15%) and translocations of ALK (5%–7%) are frequently found in Caucasian lung adenocarcinoma patients, rendering constitutive active downstream signaling of the EGFR pathway (4). These driver mutations are directly implicated in malignant processes, such as proliferation, migration, or antiapoptosis, and promote lung cancer development (5). Tyrosine kinase inhibitors are nowadays standard of care for a subgroup of metastatic NSCLC patients with activating EGFR mutations (6, 7) or ALK translocations (8). They often lead to remarkable clinical responses compared with standard cytotoxic therapy, but virtually all patients eventually relapse owing to reactivation of downstream signaling pathways (9, 10).

NSCLC cells rely on the overexpression of BCL2 and its antiapoptotic family members as part of the survival strategy during tumor progression and chemoresistance (11). In healthy cells, stress-induced intrinsic apoptosis leads to an orchestrated block of antiapoptotic BCL2 family members through BH3-only proteins, followed by mitochondrial cytochrome c-release and cleavage of caspases 9 and 3, resulting in cell death. On the other hand, upon extracellular death triggers, caspases 8 and 3 are cleaved and lead to extrinsic apoptosis. These critical events are counteracted through upregulation of BCL2 (12). Cross-activation of additional malignant pathways, such as NF-kB favor BCL2-mediated antiapoptosis. Physiologically, NF-kB is kept in check by the inhibitor of NF-kB α (IκBα), but upon stimulation by external signals or stress, IκB kinase (IKK) is activated and phosphorylates and targets IκBα for proteasome degradation. As a result, NF-kB translocates into the nucleus,
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where it transactivates genes involved in proliferation and antiapoptosis (reviewed in ref. 13).

miRNAs are small noncoding RNA molecules, which regulate biologic processes by binding target mRNAs, leading to their inactivation by translational inhibition or degradation. miRNAs participate in signaling networks as backups of transcriptional control (reviewed in ref. 14). They act either as signal amplifiers or attenuators and promote cross-talk between signaling pathways. Alterations in miRNA expression critically contribute to tumorigenesis and resistance mechanisms (reviewed in refs. 15, 16).

Reconstituting the expression of miRNAs in the tumor of patients by administering antagonimRs or by miRNA delivery may open novel avenues for personalized medicine. Similar approaches could be used to interfere with resistance mechanisms.

In this study, we aimed to identify miRNAs that act as mediators of EGFR signaling. To this end, we ecotopically expressed constitutively active KRASG12V or PIK3CAH1047R in bronchial epithelial cells and provide results showing that miR-29b is strongly upregulated in cells harboring mutated KRASG12V. We report for the first time that miR-29b is responsible for KRAS-induced protection of lung cancer cells from extrinsic apoptosis by mediating the activation of the NF-kB pathway. Interestingly, miR-29b confers sensitivity to cisplatin-induced apoptosis, suggesting that miR-29b tips the balance from extrinsic apoptosis toward intrinsic apoptosis.

Materials and Methods

Cell lines

The human NSCLC cell lines A549 and H1299 were obtained from the ATCC in 2008 and were cultured in Iscove's Modified Dulbecco's Medium (Sigma-Aldrich). Human bronchial epithelial BEAS-2B cells (kindly provided by Prof. M. Geiser, Institute of Anatomy, University of Bern, Bern, Switzerland, in 2012) were cultured in DMEM (Invitrogen). Human PC9ER and PC9 (in 2013) and H460 (in 2015) were kindly provided by Dr. A. Arcaro (Department of Clinical Research, University of Bern, Bern, Switzerland, in 2012) and H460 (in 2015) were kindly provided by Dr. A. Arcaro (Department of Clinical Research, University of Bern, Bern, Switzerland, in 2012) and H460 (in 2015) were kindly provided by Dr. A. Arcaro (Department of Clinical Research, University of Bern, Bern, Switzerland, in 2012). HCC4011 and HCC827 (kindly provided by Prof. A. F. Gazdar and Prof. J. Minna, University of Texas Southwestern Medical Center, Dallas, TX, in 2009) were cultured in RPMI medium. All cell lines were authenticated by cell line typing analysis (STR profiling) in 2016. Media were supplemented with 2 mmol/L L-alanyl-L-glutamine (Bioswisstec AG) 1% penicillin/streptomycin, and 5% to 10% FBS (Sigma-Aldrich) at 37°C and 5% CO2.

Constructs

The construct pBabe-puro containing constitutive active KRASG12V was purchased from Addgene and pBabe-TNFAlpha3 lacking the 3′ untranslated region (UTR) is described elsewhere (17). The wild-type or mutated miR-29b target sites in TNFAIP3 luciferase reporter constructs were cloned as described previously (17).

Transfections

NSCLC cells (1.5 × 10^6) were let to adhere overnight and were transfected with 50 nmol/L precursor hsa-miR-29b, pre-miR negative control #1 (Ambion), esiRNA human tnfaip3 (Sigma-Aldrich), or siGENOME nontargeting siRNA pool 2 (Dharmacon) by using the transfection reagent HiPerfect (Qiagen) as published previously (18). Ten nanograms of luciferase reporter constructs and 40 ng pGL4.74-normalizing plasmid or 25 ng pGL4.32 luciferase reporter plasmid and 1.5 ng pGL4.73-normalizing plasmid (Promega AG) were used in combination with the transfection reagent Attractene (Qiagen).

Lentiviral and retroviral transduction

Retroviral production was carried out as described previously (19). Puromycin (0.5 μg/mL; Sigma-Aldrich) was used for selecting transduced cells. Lentiviral production of the antisense construct hsa-miR-29b (pMRZIP-29b-PA-1) or scramble control (System Biosciences) was carried out as described previously (20).

Luciferase activity and apoptosis assays

Luciferase reporter assays or luciferase target validation assays were performed as described previously (17), except that 10 ng/mL TNFα (PeproTech) was used to induce the NF-kB pathway. TNFα (10 ng/mL) plus 0.5 μg/mL actinomycin D (Sigma-Aldrich), 50 ng/mL TRAIL (Genentech), or 4 μg/mL cisplatin (Spitalpharm, Bern, Switzerland) was used to induce cell death. Caspase-3/7, 8, or 9 activity was determined as described previously (17). Annexin V/propidium iodide (PI)–positive cells were analyzed using a LSR II Flow Cytometer (Becton Dickinson) and FlowJo software version 9.8.2 (Tree Star). Briefly, cells were harvested in Annexin Binding Buffer [0.01 mol/L HEPES (pH 7.4), 0.14 mol/L NaCl, 2.5 mmol/L CaCl2]. Apoptosis was assessed upon cell staining in 200 μL Annexin Binding Buffer containing Annexin V-FITC (BioLegend Inc.) diluted 1:200 and 1 μg/mL PI (Sigma-Aldrich).

RNA isolation and real-time qPCR

RNA extraction and qPCR were carried out as described previously (18). miRNA levels were analyzed using Taqman Assay (Applied Biosystems), and mRNA levels were analyzed using QuantiTect Primers (Qiagen).

miRNA array

miRNA profiling was performed by fluorescence hybridization (Trazor Industries Inc.; Exiqon A/S). The x-fold expression was determined by normalizing KRASG12V to control-transduced cells.

Cell fractionation and Western blot analysis

Cell fractionation and Western blot analysis were performed as described previously (17), except that 20 μg of protein was loaded per lane. Protein expression was determined by using antibodies detecting AKT (clone 40D4, 1:1,000), phospho-AKT (D7F10, 1:1,000), ERK1/2 (L34F12, 1:2,000), phospho-ERK1/2 (D13.14.4E, 1:2,000), phospho-S6 (polyclonal, 1:1,000), TNFAIP3 (polyclonal, 1:1,000), phospho-1/4β (5A5, 1:2,000), H3 (D1H2, 1:2,000, CST), α-tubulin (DM1A, 1:2,000, CST), CCND1 (SP4, 1:500, Dako Denmark S/A), GAPDH (D16H11, 1:2,000, CST), and p65 (polyclonal; 1:2,000). Quantification and normalization of protein levels was performed as described previously (17).
Patient’s collective

A total of 73 primary adenocarcinoma of the lung formalin-fixed paraffin-embedded (FFPE) tissue samples were retrieved randomly from the archive of the Institute of Pathology, University of Bern. Clinical–pathologic characteristics are provided in Supplementary Table S1. Tumor DNA was analyzed for KRAS and EGFR mutations by pyrosequencing or Sanger sequencing as described previously (21). Copy numbers of the miR-29b loci were assessed by qPCR using primer pairs encompassing a region from the pre-miRNA (22) relative to the autosomal reference gene GAPDH (QT02504271, Qiagen). The ethical board of the Inselspital Bern approved the study as part of the general approval for research on formalin FFPE (KEK#200/2014).

Statistical analysis

By using the unpaired two-tailed Student t test in GraphPad Prism software version 6.03 (GraphPad Software), statistical differences were calculated. Statistical significance was achieved at a probability of \( P < 0.05 \); \( *, P < 0.05; ***, P < 0.001; \), \( ***, P < 0.0001, \) ns, not significant.

Results

KRAS mutation induces upregulated miR-29b expression

The ERK and PI3K pathways constitute the major branches of the EGFR pathway in NSCLC. To identify effector miRNAs of the EGFR pathway, bronchial epithelial BEAS-2B cells were retrovirally transduced with constitutively active KRAS(G12V) or PIK3CA(H1047R) mutants, and individual clones were analyzed for the activity of signaling pathways by Western blot analysis. BEAS-2B cells were selected as they give rise to low intrinsic activity of the EGFR pathway (23). Expression of KRAS(G12V) in BEAS-2B cells resulted in up to 27-times higher ERK1/2 phosphorylation and up to 38-times higher expression of its downstream effector CCND1 relative to the control (Fig. 1A). PIK3CA(H1047R)-transduced clones revealed up to 5-fold elevated pAKT levels, but the level of the downstream effector pS6 was not elevated (Supplementary Fig. S1). miRNA expression profiling was carried out by microarrays using the Exiqon S/A and Toray Inc. platforms. Both platforms revealed enhanced levels of all miR-29 paralogs, miR-138 and miR-222 in KRAS(G12V) cells (Table 1), while no significant change in the miRNA expression profile was detected in PIK3CA(H1047R) cells (data not shown). miR-222 is a known effector of EGFR signaling in lung cancer (24), whereas miR-29 and miR-138 have not been studied yet in EGFR signaling. We validated upregulation of miR-29b and miR-138 expression by qPCR in clonal BEAS-2B cells transduced with KRAS(G12V) (Fig. 1B; Table 1). In line with these results, miR-29b expression was upregulated in primary adenocarcinoma tissue samples harboring KRAS or EGFR mutations relative to adenocarcinomas containing wild-type KRAS and EGFR (Fig. 1C; Supplementary Table S1). Moreover, we confirmed that miR-29b expression is downregulated in primary NSCLC.
with wild-type EGFR and KRAS compared with matched normal tissue (Fig. 1C; ref. 25), which was mainly due to changes in copy number of the miR-29b2 locus (Supplementary Fig. S2). However, we were unable to confirm induced miR-138 expression in mutant KRAS tumor samples, as KRAS- and EGFR-mutant and wild-type tumors gave rise to similar levels of miR-138 (Supplementary Fig. S3). We focused on miR-29b for further studies, as this paralog was most strongly induced by KRAS signaling (Table 1).

miR-29b expression is regulated via the KRAS–ERK signaling pathway

KRAS induces both ERK and PI3K signaling pathways. To elucidate the underlying regulation of miR-29b expression, we employed inhibitors of the EGFR signaling pathway and measured miR-29b expression by qPCR. First, EGFR inhibition by gefitinib led to downregulated miR-29b expression in PC9 or HCC827 cells harboring an activating EGFR mutation. In contrast, miR-29b expression was only slightly reduced in gefitinib-resistant PC9ER cells (Fig. 2A). Consistent with this finding, CCND1 mRNA levels were reduced in PC9 and HCC827 cells, but not in PC9ER cells (Supplementary Fig. S4A). The MEK inhibitor U0126 led to significantly reduced levels of miR-29b (Fig. 2B) as well as pERK1/2 (Supplementary Fig. S4B) in the NSCLC cell lines PC9 and HCC4011 harboring an EGFR mutation. Similar results were also obtained in the A549 cell line harboring a KRAS mutation. In contrast, inhibition of PI3K by LY294002 did not alter the expression of miR-29b (Fig. 2C), but pS6 protein was strongly reduced (Supplementary Fig. S4B). miR-29b expression was also significantly decreased upon MEK inhibition in KRAS-transduced as well as in control-transduced BEAS-2B cells, whereas PI3K inhibition had no effect (Fig. 2D). Thus, induction of miR-29b expression by EGFR signaling is mediated via the KRAS/MEK/ERK pathway.

NSCLC cells overexpressing miR-29b are protected from extrinsic apoptosis

KRAS is an important mediator of oncogenic processes. In lung cancer, constitutively active KRAS-mutant forms confer apoptosis resistance by inducing NF-κB signaling (26, 27). In line with this finding, we show that ectopic expression of KRASG12V conferred resistance of BEAS-2B cells to extrinsic apoptosis (Fig. 3A) induced by TNFα and actinomycin D (TNFα + ActD; ref. 28). To assess whether miR-29b is a mediator of KRAS signaling, we overexpressed precursor miR-29b in NSCLC cells and measured caspase-3/7 cleavage upon triggering apoptosis by TNFα + ActD. Indeed, A549 or BEAS-2B cells overexpressing miR-29b were protected from TNFα + ActD–induced apoptosis (Fig. 3B), thereby

Table 1. Fold induction of miRNAs in KRASG12V-transduced BEAS-2B cells

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Toray</th>
<th>qPCR</th>
<th>Exiqon</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-29b</td>
<td>3.77</td>
<td>4.72 ± 0.27</td>
<td>2.01</td>
<td>2.27 ± 0.50</td>
</tr>
<tr>
<td>miR-222</td>
<td>2.85</td>
<td>n.a.</td>
<td>1.74</td>
<td>n.a.</td>
</tr>
<tr>
<td>miR-29a</td>
<td>2.48</td>
<td>n.a.</td>
<td>1.84</td>
<td>n.a.</td>
</tr>
<tr>
<td>miR-138</td>
<td>2.34</td>
<td>2.73 ± 0.06</td>
<td>2.17</td>
<td>2.76 ± 0.45</td>
</tr>
<tr>
<td>miR-29c</td>
<td>2.31</td>
<td>n.a.</td>
<td>1.89</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

NOTE: miRNA expression was assessed by Toray Inc. and Exiqon S/A platforms. miRNA expression levels from the same samples were validated by qPCR (n = 3). Abbreviation: n.a., not analyzed.

Figure 2.

Inhibitors of the EGFR–ERK signaling pathway decrease miR-29b expression. NSCLC cell lines were treated with 1.25 μmol/L gefitinib (A) for 48 hours, 20 μmol/L U0126 (B) for 72 hours, 20 μmol/L LY294002 (C) for 72 hours, or pBABE-transduced BEAS-2B clones were treated with 10 μmol/L U0126 or LY294002 (D) for 48 hours. miR-29b expression was quantified by real-time qPCR and normalized to RNU48. Control (Ctrl) cells were treated with DMSO. ns, not significant. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
miR-29b protects NSCLC cells from extrinsic apoptosis.

miR-29b exerts the KRAS signal by activating the NF-κB pathway

miR-29b induces NF-κB activity and confers resistance to extrinsic apoptosis by targeting TNFAIP3

Database searches for miR-29b–regulated genes implicated in NF-κB signaling revealed that TNFAIP3, also known as A20, is a

### Figure 3

**A** BEAS-2B and A549 cells were treated with TNFα + ActD (A, B, D, F, and H) or 50 ng/mL TRAIL (C and G) for 6 to 12 hours or 75 minutes, respectively. Where indicated, apoptosis was induced 62 hours posttransfection. Apoptosis was measured by caspase-3/7 cleavage (A, B, C, E, and H), Annexin V positivity/PI negativity (D), or caspase-8 cleavage (F, G). Data are presented relative to control-treated cells. Pre-control (Pre-ctrl) transfected cells or the individually transduced cell lines were cultured in the absence of an apoptosis trigger were set to 100%. ns, not significant. *: P < 0.05; **: P < 0.01; ***: P < 0.001.
predicted target of miR-29b (Supplementary Fig. S6A). It is induced by NF-κB and acts in a negative feedback loop to block NF-κB activity. Reduced expression of TNFAIP3 promotes cancer development (reviewed in ref. 31) as this leads to constitutive activation of NF-κB signaling. TNFAIP3 expression is frequently downregulated in lung cancer (www.proteinatlas.org), but the underlying molecular mechanism is unknown. To assess whether TNFAIP3 is a target of miR-29b, a fragment from the 3’ UTR of
TNFAIP3 encompassing the predicted miR-29b-binding site was cloned downstream of the luciferase gene and transiently transfected into A549, H1299, H460, or BEAS-2B cells. The wild-type miR-29b target site conferred reduced luciferase activity relative to the progenitor construct containing no target site (n = 5). B-D, Western blot analysis of NSCLC cells transfected with pre-miR-29b (B) or transduced with KRASG12V (C) or anti-miR-29b constructs (D) using a TNFAIP3-specific polyclonal antiserum. Protein levels were normalized to GAPDH and presented relative to the control. E, NF-κB activity (top) and apoptosis (bottom) of cells transduced with a TNFAIP3 expression construct lacking the miR-29b target site (TNFAIP3) or control vector (pBABE). ns, not significant. Cells were transfected with pre-miR-29b or pre-control (Pre-ctrl) and analyzed 2 to 3 days posttransfection. F, NF-κB activity (top) and apoptosis (bottom) of cells transfected with siRNA pool targeting TNFAIP3/C3. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
miR-29b elicits a 1.4- to 1.5-fold induction of NF-κB activity in control transduced A549 or BEAS-2B cells, whereas NF-κB induction was completely abrogated in cells ectopically expressing TNFAIP3 (Fig. 5E). In line with these results, miR-29b was significantly less efficient in conferring apoptosis resistance in A549 or BEAS-2B cells ectopically expressing TNFAIP3 compared with control-transduced cells. Thus, ectopic TNFAIP3 can restore miR-29b-induced activation of NF-κB and apoptosis resistance. In agreement with these results, a siRNA pool targeting TNFAIP3 phenocopied enhanced NF-κB activity and reduced caspase-3/7 activity elicited by miR-29b (Fig. 5F and Supplementary Fig. S6F and S6G).

**miR-29b discriminates between mode of apoptosis**

Interestingly, miR-29b is a known tumor-suppressing miRNA that induces apoptosis in different cell systems (32). This is in contrast to our findings that miR-29b protects NSCLC cells from extrinsic apoptosis. To address these apparently contradictory findings, A549 or H1299 cells were treated with cisplatin, which induces mitochondrial apoptosis by activating stress-sensing proapoptotic BCL2 family members. To our surprise, miR-29b overexpression conferred apoptosis sensitivity rather than resistance under these conditions, as indicated by an up to 3-times higher caspase-3/7 activity (Fig. 6A) and a 4.4-times higher proportion of Annexin V-positive cells (Fig. 6B and Supplementary Fig. S7A). In contrast, caspase-3/7 activity was reduced in A549 and H1299 cells transduced with anti-miR-29b (Fig. 6A). Furthermore, cisplatin-induced caspase-8 as well as caspase-9 activities were increased (Fig. 6C and D) while MCL1 mRNA was decreased (Supplementary Fig. S7B) upon miR-29b overexpression, supporting the notion that miR-29b sensitizes cells to cisplatin-induced apoptosis via both apoptotic pathways. In comparison, diminished caspase-9 activity after TNFα + ActD administration was detected in Fig. 6D, indicating that miR-29b facilitates TNFα + ActD–induced apoptosis via caspase-9, which is typically found in type II–specific cell death (33).

**Discussion**

A growing number of miRNAs may exhibit a dual function as a tumor-suppressing miRNA and an oncogenic miRNA depending on the tissue and cellular context. miR-29b sensitizes cancer cells to apoptosis by targeting antiapoptotic genes, including MCL1, BCL2, AKT2, CCND2, and CDC42 (reviewed in ref. 32). It also negatively regulates proliferation and migration by targeting genes involved in the regulation of the extracellular matrix and restores erroneous promoter methylation by targeting DNMT3A and 3B. On the other hand, miR-29b or its paralogs promote tumor progression, apoptosis resistance, and metastasis of hematologic malignancies and solid cancers by targeting key tumor suppressors, including PTEN, TET2, or proapoptotic genes (34–36). The molecular mechanisms that determine whether a miRNA is tumorigenic or antitumorigenic are largely unknown. We show that miR-29b can act in a proapoptotic or antiapoptotic manner depending on the external stimulus (Fig. 6E). Consistent with results in other cell systems (37), we show that miR-29b sensitizes NSCLC cells to cisplatin-induced apoptosis. Cisplatin is considered primarily an inducer of intrinsic apoptosis, although extrinsic apoptosis is also induced to some extent (38). In agreement with this finding, both caspase-9 and caspase-8 activities were significantly enhanced by miR-29b. In contrast, extrinsic apoptosis induced by TNFα + ActD or TRAIL was significantly reduced in NSCLC cells overexpressing miR-29b. Thus, miR-29b tips the balance from extrinsic to intrinsic apoptosis. This is a novel finding, which may have profound consequences for the biology of tumor cells (see below).

miR-29b confers apoptosis resistance by targeting TNFAIP3/ A20. This is based on the finding that miR-29b affects the expression of TNFAIP3 protein and represses the activity of a luciferase reporter construct containing the miR-29b target site. In agreement with this result, NF-κB activity was significantly induced in cells overexpressing miR-29b as indicated by NF-κB reporter assays, IκB phosphorylation, and nuclear translocation of NF-κB as well as induced expression of NF-κB–responsive genes. Intra- cellular miR-29b levels were sufficient to affect NF-κB activity as
indicated by KRASG12V/anti-miR-29b cotransduction experiments. Most importantly, however, enhanced NF-kB activity and reduced apoptosis elicited by miR-29b was abrogated in NSCLC cell lines overexpressing miR-29b–refractory TNAFAP3. Thus, TNAFAP3 is a relevant target of miR-29b implicated in these processes. NF-kB activity induced by miR-29b leads to upregulation of antiapoptotic genes, rendering cancer cells insensitive to the extrinsic apoptotic program (39). Expression of BCL2 family members is also induced by NF-kB conferring insensitivity to cisplatin (40). However, it is well established that miR-29b directly targets the antiapoptotic gene MCL1 in lung cancer (41), tipping the balance toward intrinsic apoptosis (Fig. 6E). miR-29b was previously shown to act as a decoy to protect TNAFAP3/A20 from degradation by HuR in sarcomas, which is based on the finding that miR-29b directly interacted with HuR mRNA, but not with TNAFAP3 mRNA (42). The reason for the discrepancy of these results is unknown, but it may be explained by differences of HuR mRNA expression in these tumor systems.

We show for the first time that miR-29b is an important mediator of KRAS signaling in antiapoptotic processes of NSCLC. Overexpression of oncogenic KRAS results in NF-kB activation (26, 43–46), whereas inhibition of NF-kB signaling induced apoptosis (26, 46), underlining the importance of this survival pathway in KRAS-induced transformation. However, the underlying molecular mechanism of KRAS-induced NF-kB activation is not well understood. It was suggested that KRAS induces NF-kB activation by phosphorylation of IKK (43), but this was contradicted by others (44, 45). We confirmed enhanced NF-kB activity in lung epithelial cells overexpressing KRASG12V. In addition, we provide a novel mechanism by which miR-29b links the KRAS signaling with the NF-kB pathway. We show that miR-29b expression is significantly induced in epithelial cells overexpressing KRASG12V as well as in NSCLC tissues harboring KRAS or EGFR mutations. In agreement with these results, inhibitors of EGFR and MEK, but not PI3K, reduced the expression of miR-29b. To link KRAS and NF-kB signaling, we downregulated miR-29b expression in KRASG12V–transduced cells and demonstrated that NF-kB activity was completely restored. In agreement with this result, cells were more susceptible to extrinsic apoptosis, confirming that apoptosis resistance was due to enhanced NF-kB activity. KRAS-transduced tumors rely on NF-kB activity for survival (25, 46). No significant correlation of the postoperative overall survival and miR-29b expression was noted for patients with KRAS or EGFR mutations (data not shown). This may be due to the heterogeneous collective in terms of clinical–pathologic parameters or, alternatively, may suggest an additional mechanism to miR-29b overexpression conferring apoptosis resistance of KRAS-transduced tumors.

EGFR mutations (5, 6) and ALK or ROS translocations (8), which occur in about 20% of NSCLC, were successfully exploited for the development of targeted therapy. In contrast, patients with KRAS mutations, which occur in 20% to 30% of NSCLC, are not amenable to novel forms of therapy. KRAS initiates an apoptotic response, which is suppressed by the activation of NF-kB in cell lines (46) as well as in a mouse model of lung cancer (26). Thus, NF-kB inhibition could be exploited for the treatment of KRAS-driven lung adenocarcinoma. This could potentially be achieved by administering a miR-29b antagonist, as this may uncouple KRAS and NF-kB signaling. On the other hand, miR-29b overexpression may sensitize NSCLC to platinum-based therapy. It should be kept in mind, however, that a miR-29b–based therapy may turn out to be a double-edged sword, as it may interfere with both apoptotic pathways in opposite directions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: S. Langsch, U. Baumgartner, S. Haemmig, M.P. Tschan, E. Vassella
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Langsch, U. Baumgartner, S. Haemmig, C. Schlup, S. Berezowska, G. Rieger, P. Dorn, E. Vassella
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Langsch, U. Baumgartner, S. Haemmig, S.C. Schäfer, G. Rieger, P. Dorn, E. Vassella
Writing, review, and/or revision of the manuscript: S. Langsch, U. Baumgartner, S.C. Schäfer, S. Berezowska, P. Dorn, M.P. Tschan, E. Vassella
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Langsch, U. Baumgartner, C. Schlup, S. Berezowska, E. Vassella
Study supervision: E. Vassella

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References
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Correction: miR-29b Mediates NF-κB Signaling in KRAS-Induced Non-Small Cell Lung Cancers

In this article (Cancer Res 2016;76:4160–9), which appeared in the July 15, 2016 issue of Cancer Research (1), the labels on the right panel of Fig. 3B are incorrect due to publisher error and should read "Pre-ctrl" and "Pre-miR-29b."

In addition, Ulrich Baumgartner should be included in the Authors' Contributions section under "Acquisition of data."

The online version of the article has been corrected and no longer matches the print.

Reference

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