Selective requirement for Mediator MED23 in Ras-active lung cancer

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K-RAS-activating mutations occur frequently in non-small cell lung cancer, leading to aberrant activation of the Ras-MAPK signaling pathway that contributes to the malignant phenotype. However, the development of Ras-targeted therapeutics remains challenging. Here, we show that MED23, a component of the multisubunit Mediator complex that is known to integrate signaling and gene activities, is selectively important for Ras-active lung cancer. By screening a large panel of human lung cancer cell lines with or without a Ras mutation, we found that *Med23* RNAi specifically inhibits the proliferation and tumorigenicity of lung cancer cells with hyperactive Ras activity. *Med23* deficiency in fibroblasts selectively inhibited the oncogenic transformation induced by Ras but not by c-Myc. The transcription factor ELK1, which is phosphorylated by MAPK for relaying Ras signaling to MED23, also was required for the Ras-driven oncogenesis. Transcriptome analysis revealed that MED23 and ELK1 co-regulate a common set of target genes enriched in regulating cell-cycle and -proliferation functions to support the Ras dependency. Furthermore, MED23 was up-regulated by Ras transformation in correlation with the strength of Ras signaling as indicated by the ELK1 phosphorylation level and was found to be overexpressed in both Ras-mutated lung cancer cell lines and primary tumor samples. Remarkably, lower *Med23* expression predicted better survival in Ras-active lung cancer patients and xenograft mice. Collectively, our findings demonstrate a critical role for MED23 in enabling the “Ras-addiction” of lung carcinogenesis, thus providing a vulnerable target for the treatment of Ras-active lung cancer.

oncogene addiction | transcription cofactor | therapeutic target

Lung cancer is the most common form of cancer globally, with an estimated 1.2 million new cases each year. It is also the leading cause of cancer-related mortality worldwide, with a median survival time of 8–11 mo and a 5-y survival rate of 15% (1). The high mortality rate of this disease results primarily from the diagnosis of the majority of lung cancers at advanced stages when the standard treatment options, such as traditional cytotoxic chemotherapy, are mostly palliative and confer only a limited survival benefit (2). Therefore, a better understanding of the molecular origins and evolution of lung cancer is needed to develop novel treatment strategies.

As with most cancers, lung cancer arises as a consequence of the accumulation of multiple oncogenic lesions. A point mutation in the *K-Ras* gene and aberrant Ras-MAPK pathway functioning are early events that contribute to the malignant phenotype of some types of lung cancer (3). *Ras* gene family members are mutated in ~30% of human tumors, and oncogenic mutations in the *K-Ras* gene are present in ~35% of non-small cell lung cancers (NSCLCs) (4, 5). Despite its prominent status as an attractive drug target, development of therapeutics aimed at disrupting the active-Ras function has proven challenging thus far (6, 7). One obstacle to the development of specific Ras inhibitors is the tendency of mutated RAS proteins to gain constitutive activity, having lost their normal enzymatic function of switching between the active, GTP-bound state and the inactive, GDP-bound state (8). Such loss-of-function enzymes are much more difficult to inhibit than gain-of-function

activated enzymes, such as those produced by EGFR mutations. Moreover, *K-Ras* mutations have been shown to confer primary or de novo resistance to EGFR-targeted therapies (9). Although therapeutics targeting the downstream effector molecules of Ras, such as Raf, MEK, and PI3K/mTOR, have proven efficacious in treating tumors with Ras mutations, acquired drug resistance invariably evolves during the treatment (10–13). Consequently, the need to identify additional, pharmacologically tractable components for K-Ras-driven tumorigenesis remains pressing.

The Mediator complex is a multisubunit coactivator complex that is evolutionarily conserved from yeast to mammal and that can act as a molecular bridge between gene-specific transactivators and the RNA polymerase II-associated basal transcription machinery (14, 15). Through physical interactions between the various transcription factors and specific Mediator subunits, the Mediator complex functions as an integrative hub for channeling different signaling pathways (16), such as the nuclear hormone receptor pathway (via MED1) (17), the TGF- β -signaling pathway (via MED15) (18), the Wnt-signaling pathway (via MED12) (19), and the Ras-MAPK signaling pathway (via MED23) (20, 21). Emerging studies are beginning to focus on the specificity of Mediator in regulating diverse biological processes, including differentiation, proliferation, metabolism, and carcinogenesis.

Mediator subunit MED23 (*Sur2*) was identified originally as a genetic regulator of hyperactive Ras in *Caenorhabditis elegans*. The loss of *Med23* could block abnormal vulval development, the phenotype exhibited in response to activated Ras (22). Considering MED23 as a downstream regulator for the Ras-MAPK signaling pathway, we investigated the function of MED23 in Ras-active lung cancer and explored whether it could be used for clinical diagnosis and target in lung cancer therapy. We found that MED23 and its binding partner Ets-like protein-1 (ELK1) are the critical regulators of “Ras-addicted” lung cancer. Moreover, the expression level of MED23 is correlated with the level of Ras or MAPK activity in human lung cancers and is associated with the prognoses of patients who have Ras-active lung cancer. These findings demonstrate a selective role for MED23 in supporting Ras addiction and Ras-active lung cancer and suggest that MED23 might be a therapeutic target in Ras-active lung cancer.

Results

Med23 Depletion Selectively Inhibits Proliferation and Tumorigenicity of Lung Cancer Cell Lines Carrying Ras Mutations. To determine the function of *Med23* in lung cancer cells, we used retrovirus-based

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shRNA to knock down *Med23* expression in A549, an NSCLC cell line that harbors a mutated *K-Ras* gene. Three different shRNAs effectively attenuated the expression of MED23 in A549 cells as indicated by the immunoblotting results, whereas a negative control shRNA did not affect the MED23 expression (Fig. 1A).

We first investigated whether MED23 is involved in growth control. Equal numbers of WT A549 cells, cells expressing a control shRNA (si-Ctrl), and cells expressing an shRNA against *Med23* (si-Med23) were cultured for 6 d. As shown in Fig. 1B, *Med23* knockdown decreased the proliferation of all of the si-Med23 cells compared with the parental cells and the control shRNA cells. To exclude the off-target effects of the shRNA, the mouse *Med23* gene, which is resistant to the shRNA specific for the human *Med23*, was reintroduced into the si-Med23 A549 cell lines using retroviral transduction (Fig. S1A). Cells with the incorporated gene were selected with hygromycin. The cell-proliferation assay revealed that ectopic expression of mouse *Med23* can rescue the growth defect of the si-Med23 A549 cells (Fig. S1B). These data showed that the shRNA's effect is on target. We next investigated whether MED23 participates in anchorage-independent growth and tumorigenesis of A549 cells. Using a soft agar colony-formation assay, we found that the loss of MED23 impaired A549 colony formation in a semisolid medium, and the quantitation of the colonies revealed that *Med23* knockdown not only reduced the colony size but also decreased the number of colonies (Fig. 1C and Fig. S1E and F). To confirm these observations in vivo, equal numbers of si-Ctrl or si-Med23 A549 cells were implanted s.c. into athymic nude mice. After 7 wk, mice injected with the si-Med23 cells showed smaller tumor masses and volumes than mice injected with si-Ctrl cells (Fig. 1D). These findings suggest that MED23 plays an important role in tumorigenicity, because the depletion of *Med23* inhibited the proliferation and tumorigenicity of A549 cells. Through FACS analysis, we also checked whether *Med23* depletion causes increased apoptosis. We observed no increase in annexin V-positive staining cells after *Med23* knockdown (using cisplatin-treated si-Ctrl cells as a positive control), suggesting that *Med23* knockdown does not induce cell death (Fig. S2). Thus, the decrease in lung cancer cell numbers and tumorigenicity is the result of the change of the proliferation rate by *Med23* knockdown.

We then expanded our screening survey to a larger panel of human lung cancer cell lines with or without a Ras mutation to explore further the phenomenon of *Med23* dependency in growth control. The Ras-mutation status of the various cancer cells used in this study was verified using the COSMIC database, and the Ras dependency of these cells was determined previously using high-throughput RNAi (23, 24). Remarkably, the depletion of *Med23* selectively inhibited the proliferation of the cells with mutated Ras, such as H1299, HTB-177, and CRL-5807 (Fig. 1E); in contrast, the *Med23* knockdown did not appear to affect the growth of the cells with WT Ras, such as HTB-182, H522, and CRL-5889 (Fig. 1F). Similar results using these lung cancer cells with or without the Ras mutation were observed for the processes of anchorage-independent growth and tumor formation in nude mice (Fig. S3A). These results suggest a close relationship between the presence of an activated Ras mutation and the requirement for MED23 to control the growth of lung cancer cells.

To test the idea that the Ras-active mutation indeed couples with MED23-dependent growth control, we introduced the oncogenic HRasV12 allele into two lung cancer cell lines with WT Ras, HTB-182, and H522 via retroviral transduction. Notably, after expressing the oncogenic Ras, both cell lines, which previously were resistant to *Med23* depletion, displayed dependence on MED23 to some extent; i.e., their growth rates became partially inhibited by *Med23* depletion (Fig. S3B). This result further supported the idea that MED23 plays an important role in the controlling the growth of the lung cancer cells in a Ras activity-dependent manner.

Fig. 1. MED23 is selectively important for the proliferation and tumorigenicity of Ras-active lung cancer cell lines. (A) Western blot analysis of lysates from control and si-Med23 A549 cells shows the knockdown effect of *Med23* expression. si-Med23 A, si-Med23 B, and si-Med23 C indicate three different oligos that target *Med23*. TATA Box Binding Protein (TBP) was blotted as loading control. (B) Cell-proliferation assay following retroviral shRNA-mediated *Med23* ablation in A549 cells. WT, si-Ctrl, and si-Med23 (A, B, and C) A549 cells were plated in 12-well plates, and cell numbers were determined every day until day 6. All cell-proliferation assays were done in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; error bars indicate SD. (C) Soft agar growth assay of si-Ctrl and si-Med23 A549 cells. Representative photographs of soft agar colony formation at day 18 are shown. The colony was stained with 1 mg/mL MTT for 6 h. (Scale bars: 2.0 mm.) (D) Tumor-formation assays of si-Ctrl and si-Med23 A549 cells in athymic nude mice. Mice injected with si-Ctrl and si-Med23 cells are shown, and the tumors that developed at injection site are shown below the corresponding mice. Graphs display the average tumor mass. *** $P < 0.001$; error bars indicate SD. (E) Effect of *Med23* knockdown on the proliferation of the Ras-active cancer cells. HTB-177 and CRL-5807 are lung cancer cell lines with K-Ras mutation; H1299 is a lung cancer cell line with an N-Ras mutation; PANC-1 is a pancreatic cancer cell line with a K-Ras mutation. (Upper) Cell number was counted at day 6 and was normalized to control cells. ** $P < 0.01$, *** $P < 0.001$; error bars indicate SD. (Lower) Western blot (WB) analysis of lysates from these cells following retroviral-mediated *Med23* knockdown shows the effects on MED23 expression. (F) Effect of *Med23* knockdown on the proliferation of cancer cells without Ras mutation. H522, HTB-182, and CRL-5889 are lung cancer cell lines; DU145 is a prostate cancer cell line.

To test the applicability of the correlation between Ras-mutation status and sensitivity to *Med23* depletion to other types of cancer, we investigated the effects of *Med23* depletion in different epithelial cancer cell lines. Consistent with our observations in the lung cancer cell lines, the depletion of *Med23* impaired the growth of Ras-active pancreatic cancer PANC-1 cells, but not prostate cancer DU145 cells that carry a WT Ras (Fig. 1 *E* and *F*). Taking these results together, we found that, in general, the growth of Ras-active cancer cells is more sensitive to MED23 deficiency than cancer cell lines with WT Ras. These observations strongly suggest that MED23 is selectively required for the growth of Ras-active cancer cells.

MED23 Is Required for Ras- but Not Myc-Induced Cell Transformation.

Because MED23 was found to be important for the proliferation and tumorigenesis of Ras-active cancer cells, we next attempted to establish the functional relationship between MED23 and Ras during oncogenesis. Active Ras is known to be a robust oncogene in inducing malignant transformation (4). We asked whether MED23 is required in Ras-induced transformation. The expression of *Ras* alone in primary mouse embryonic fibroblasts (MEFs) induces a senescence-like arrest of growth (25, 26). However, immortalized MEFs defective in the p53 or Rb tumor suppressor pathway can be transformed by a hyperactive Ras mutant, resulting in anchorage-independent growth and tumor formation in nude mice (27, 28). Hyperactive HRasV12 was overexpressed in WT and *Med23*^{-/-} immortalized MEFs through viral transduction (Fig. 2*A*). The HRasV12 expression in WT MEFs induced anchorage-independent colony growth and tumor formation in the immunodeficient mice; however, Ras transformation was much less efficient in the KO (*Med23*^{-/-}) MEFs than in the WT MEFs (Fig. 2*B–D*). These observations confirmed that MED23 is critical for Ras to induce transformation and corroborated the role of MED23 in Ras-dependent cancer.

To determine the specific role of MED23 in Ras transformation, we performed a transformation assay using another oncogene, *c-myc*. Interestingly, Myc transformation appears to be independent of MED23. Unlike Ras transformation, the presence or absence of MED23 did not affect the efficiency of Myc transformation in terms of the size or number of anchorage-independent colonies in a soft agar colony-formation assay (Fig. 2*E* and *F*). These data indicate that there is a selective requirement for MED23 during Ras transformation and further support the codependency of active Ras and MED23 in Ras-driven oncogenesis.

ELK1 Controls Tumorigenicity of Lung Cancer Cells and Ras Transformation. ELK1 is a member of the ternary complex factor family (29). Upon activation by MAPK, phosphorylated ELK1 (p-ELK1) interacts with the Mediator complex via the MED23 subunit to stimulate the expression of downstream target genes (21, 30, 31). Therefore, ELK1 could be considered as being genetically upstream of MED23. Because the MED23 seems to control specifically the growth of tumor with activated Ras, we next asked whether ELK1 also can control the process of transformation and the tumorigenicity of lung cancer cells in a Ras-specific manner.

Three different *Elk1*-specific shRNAs were expressed stably in A549 cells. Attenuated proliferation rates and tumorigenicity were observed, as indicated by the decreased numbers of cells and soft agar colonies compared with those of the control cells (Fig. 3*A–C*). Interestingly, similar to the effect of *Med23* knockdown, *Elk1* knockdown did not affect the growth of the lung cancer cells with WT Ras, including H522 and HTB-182 cells (Fig. S4*A* and *B*). We also performed the Ras-transformation assay in the immortalized MEFs with or without *Elk1* depletion and found that the *Elk1* RNAi also can decrease the efficiency of Ras transformation (Fig. 3*D–F*). These results suggest that *Elk1* seems to phenocopy *Med23* in regulating tumorigenicity and that the

Fig. 2. MED23 is essential for the Ras transformation but not for the c-Myc transformation. (A) Western blot analysis of lysates from WT and *Med23*^{-/-} (KO) MEFs with or without Ha-RasV12 overexpression. TBP was blotted as loading control. (B and C) Ras-transformed WT and KO MEFs were seeded in six-well plates in soft agar to detect the anchorage-independent growth. After 18 d, the colonies were stained with MTT and imaged. Representative fields (B) and the whole plates (C) of soft agar colonies formed by the indicated MEFs are shown. (Scale bars: 500 μ m.) (D) Tumor formation in athymic nude mice by the Ras-transformed WT and KO MEFs. Mean tumor mass of the s.c. tumors formed by WT or KO MEFs in the immunodeficient mice is shown. ****P* < 0.001; error bars indicate SD. (E) Western blot analysis of lysates from WT and KO MEFs with or without c-Myc overexpression. TBP was blotted as the loading control. (F) Myc-transformed WT and KO MEFs were seeded in six-well plates in soft agar to detect anchorage-independent growth. After 18 d, the colonies were stained with MTT and imaged.

functional interaction between MED23 and ELK1 might be responsible for the observed Ras-driven oncogenicity.

MED23 and ELK1 Coregulate a Set of Genes Related to Proliferation and Cell-Cycle Functions. Given that MED23 controls the highly specialized active Ras–MAPK signaling by interacting with ELK1, we asked whether MED23 regulates the expression of ELK1 target genes in Ras-active lung cancer cells. Because most ELK1 target genes, such as *Egr1*, *Egr2*, *Ier2*, and *c-fos*, are immediate early genes (32), we first performed a time course of serum stimulation to examine their expression patterns. The loss of MED23 greatly reduced the expression of all of the examined ELK1 target genes following serum stimulation (Fig. 4*A*). Moreover, the expression of the compromised ELK1 target genes in si-Med23 A549 cells could

MED23 Expression Correlates with Ras–MAPK Signaling Strength in Human Lung Cancers. The requirement for MED23 during Ras transformation implies a possible oncogenic role for *Med23* in Ras-dependent cancers. To understand better the function of MED23 in Ras-active lung cancer, we examined the MED23 expression in 12 human lung cell lines, including two normal human embryonic lung fibroblast lines, two immortalized normal human lung epithelial cell lines, six lung cancer cell lines with mutated Ras, and two lung cancer cell lines with WT Ras. In general, the expression of MED23 was higher in the lung cancer cells than in the normal cells. Notably, the cancer cell lines with mutated Ras expressed more MED23 and ELK1 than the cancer cell lines without a Ras mutation; thus the activation of the Ras signaling pathway correlated positively with a higher level of p-ERK, p-AKT, and p-ELK1 (Fig. 5A and Fig. S6A). Furthermore, we found that growth of the lung cancer cells with an oncogenic Ras mutation seems to be more sensitive to inhibition by the MAPK inhibitor U0126 (Fig. S6B), indicating that Ras-active lung cancer cells with higher MED23 expression are more

Fig. 4. Alteration in global gene expression following *Med23* or *Elk1* knockdown. (A) Quantitative RT-PCR analyzed the expression of several ELK1 target genes following *Med23* knockdown. si-Ctrl and si-Med23 A549 cells were serum starved overnight and then were cultured with growth medium with 20% serum for an additional 30 or 90 min. The cells were harvested and processed for quantitative PCR analyses using PCR primers specific for *Egr1*, *Egr2*, *ler2*, and *c-fos*, respectively. (B) The control of ELK1 target genes expression by MED23 is Ras dependent. Quantitative RT-PCR analyses of the expression of several ELK1 target genes in HTB-177, CRL-5807 (mutated Ras), HTB-182, and H522 (WT Ras) cells, with or without *Med23* knockdown. All the cells were serum stimulated for 30 min before collection. (C) Global changes in gene expression are similar following *Med23* or *Elk1* knockdown. Expression of many genes changed following *Med23* knockdown. Expression is ranked based on the fold change of the expression level. (Upper) "Positive" indicates genes down-regulated by *Med23* Knockdown. (Lower) "Negative" indicates genes up-regulated by *Med23* Knockdown. The correlation coefficient of the gene-expression patterns regulated by MED23 and ELK1 is 0.928. (D) Heat map showing clustering of genes with log₂ fold change > 0.5 following knockdown of *Med23* or *Elk1*. Genes were sorted by fold change relative to si-Ctrl ($P < 0.05$). (E) GO analysis of the positive-overlap genes following *Med23* or *Elk1* knockdown. "Positive overlap" indicates a set of genes down-regulated after knockdown of *Med23* or *Elk1*. Level 3 in biological process is shown in the graph, and all the top10 processes are significantly enriched ($P < 0.001$). Ranking is based on the number of changed genes. (F) Quantitative PCR confirmed the changes in the genes related to cell cycle or cell proliferation selected from the microarray analysis in E.

reliant on the hyperactive Ras-MAPK pathway. These data indicate that expression of MED23 is up-regulated specifically in lung cancer cell lines and that this up-regulation is closely associated with the Ras-mutation status of the cells. Considering that *Med23* depletion selectively inhibited Ras-active cancer cell growth, it seems that MED23 is required for Ras addiction of lung cancers. On the other hand, the MED1 expression pattern was different from that of MED23, underlining the specific role of MED23 in Ras-active lung cancers (Fig. S74).

We also evaluated MED23 expression in human lung cancer tissues to determine whether the expression level of MED23 is correlated with lung carcinogenesis *in vivo*. The expression of MED23 was assessed using a tissue microarray with 188 NSCLC samples subjected to immunohistochemistry with a MED23 monoclonal antibody (Fig. 5B). The clinical samples contained two different NSCLC subtypes: adenocarcinoma ($n = 95$) and squamous cell carcinoma ($n = 93$). A lung cancer section and the corresponding adjacent lung tissue were obtained from each

Fig. 5. MED23 is overexpressed in lung cancer with hyperactive Ras signaling. (A) Immunoblot analysis of MED23, ELK1, and p-ELK1 levels in normal embryonic lung cells (WI-38 and MRC-5), immortalized normal bronchial epithelium cells (CRL-2078 and CRL-2741), lung cancer cell lines with mutated Ras (A549, H1299, HTB-177, CRL-5807, HTB-57, and H2122) and lung cancer cell lines with WT Ras (H522 and HTB-182); β -actin was blotted as loading control. (B) Immunohistochemistry (IHC) staining of MED23 in the representative lung cancer tissues and the adjacent normal tissues. (Scale bars: 200 μ m.) (C) Immunohistochemical analysis of MED23, p-ERK, and p-ELK1 levels in consecutive sections of human lung cancer tissue array. (Scale bars: 200 μ m.) (D) Statistical analysis of the correlation between the MED23 expression and the p-ERK or p-ELK1 levels in lung cancer tissues. The cancer tissues were divided into two groups, higher staining and lower staining, based on the staining index of p-ERK (red) or p-ELK1 (blue). Then the MED23 staining index in the two groups was compared and showed good correlation with the p-ERK or p-ELK1 level ($P < 0.05$, group t test).

patient, allowing us to compare MED23 levels in normal versus neoplastic epithelium. In contrast to the adjacent normal tissues, most of the NSCLC samples from both adenocarcinomas and squamous cell carcinomas stained positive for MED23, which was clearly present in the epithelial cell nuclei. To quantify the differences between the normal and cancerous tissues objectively, a scoring system was implemented to analyze the immunohistochemical staining. Each section was scored using a staining index (33) composed of the intensity of the staining on a scale of 0–3 and the extent of the staining on a scale of 0–3. The final score of each sample was determined by multiplying the extent score by the intensity score; therefore, the final staining index ranged from 0 (no staining) to 9 (strong and extensive staining). Most of the adjacent normal tissue samples (96/188) were negative (0) for MED23, but nearly half of the NSCLC samples (91/188) exhibited a higher MED23 staining index (4–9). Indeed, 82.4% (155/188) of the cancerous tissues from the patients showed stronger staining than the surrounding normal epithelium ($P < 0.0001$, $n = 188$) (Fig. 5B and Fig. S7B). Furthermore, MED23 expression levels did not differ between the adenocarcinoma and squamous cell carcinoma samples. These

findings suggest that NSCLC tissues tend to have high MED23 expression and are consistent with our observations that MED23 is overexpressed in lung cancer cell lines.

Because MED23 expression levels were strongly associated with the Ras activity levels in lung cancer cell lines, we next asked if a similar correlation occurs in clinical NSCLC samples. Using the same NSCLC tissue array, consecutive sections of human lung cancers were stained for p-ERK and p-ELK1, because the levels of p-ELK1 and p-ERK are indicative of activated Ras signaling. Interestingly, we found that higher MED23 expression in the NSCLC samples correlated with higher p-ERK and p-ELK1 staining signals (Fig. 5C). For statistical analysis the samples were divided into two groups (the higher half versus the lower half), based on their ERK and ELK1 phosphorylation levels. Higher levels of both p-ERK and p-ELK1 correlated with higher MED23 expression levels ($P < 0.05$) (Fig. 5D). Taken together, these findings provide direct evidence that the elevated expression of MED23 in the clinical NSCLC samples is associated with activated Ras–MAPK signaling.

Because MED23 is required for the Ras transformation, and because the elevated MED23 expression in lung cancer cell lines

and clinical samples correlated with hyperactive Ras activity, we wondered whether the expression of MED23 could be regulated by oncogenic Ras during Ras transformation. To exclude the possible change of MED23 expression during immortalization, we used primary MEFs to perform the transformation assay. In contrast to transforming immortalized MEFs, transforming primary MEFs requires multiple cooperating oncogenes (34). Coexpression of Ras along with an immortalizing oncogene such as adenoviral E1A can easily induce a transformation phenotype in primary MEFs (35, 36). Significantly, transformation of primary MEFs with the combination of Ras and E1A drives up both mRNA and protein levels of *Med23* to a greater extent than seen with E1A alone or with E1A plus other oncogenes such as c-myc or T-antigen (Fig. S8A and B). Similarly, the activation of ERK and ELK1 also is highest in the Ras plus E1A transformation (Fig. S8A). Interestingly, we observed that MED23 is not the only subunit that was up-regulated in the process of Ras transformation; many of the Mediator subunits examined with the available antibodies also were up-regulated during the Ras transformation (Fig. S8C). The whole Mediator complex probably is up-regulated to meet the demand for faster proliferation; however, the MED23 subunit was specifically required for sensing and transducing the signal strength of the hyperactive Ras pathway. To demonstrate further the specificity of MED23 in Ras-driven tumorigenicity, we performed an important control experiment by knocking down another Mediator subunit, Med15. Although MED15 also showed elevated protein level during Ras transformation, Med15 knockdown failed to change the efficiency of Ras transformation or the growth rate of lung cancer cells with activated Ras activities (Fig. S9A, C, and D). Consistently, in contrast to MED23, MED15 protein levels show no correlation with the hyperactive Ras signaling in samples from lung cancer patients (Fig. S9B). These observations further underscore the critical and specific role of MED23 in Ras signaling and oncogenesis.

Med23 Expression Is Associated with Prognosis of Patients with Ras-Active Lung Cancer. Given that *Med23* is an important regulator of Ras-driven oncogenesis, patients bearing tumors with activated Ras would be expected to benefit from reductions in *Med23* expression. To test this hypothesis, we analyzed whether the expression level of *Med23* was associated with patient prognosis in a large cohort of lung cancer samples. In a previous study, a Ras-expression signature that included 766 genes first was derived from a microarray analysis of a small set of clinical lung tumor samples with known Ras mutation (37). The signature genes were used as probes in an additional set of expression profiles from 442 human lung adenocarcinomas to stratify the samples as having positive, negative, or neutral Ras signatures (38). Ultimately, researchers identified 143 tumors as having strong Ras mutant signatures (Ras + signature) and 116 as having WT Ras signatures (Ras – signature).

Given the important role of *Med23* in Ras-active lung cancer, we asked whether the expression level of *Med23* in the clinical sample was associated with the patient's prognosis in a manner that was dependent on the tumor's Ras signature status. The lung cancer patients were separated into two groups based on the expression level of *Med23* (higher half vs. lower half of *Med23* expression). The higher group (designated by *Med23* expression levels in the tumors that were higher than the median) was compared with the lower group (containing the rest of the cancer patients in the subset), using Kaplan–Meier analysis. Strikingly, we found that a lower level of *Med23* expression was associated specifically with enhanced survival for the set of patients (143) who bore tumors with a positive Ras signature ($P = 0.02$); in contrast, the *Med23* expression level had no predictive value for the set of patients (116) who bore tumors with a negative Ras signature ($P = 0.84$) (Fig. 6A and B). As a control, the expression

of another Mediator subunit, Med15, did not associate with the prognoses of Ras-active lung cancer patients (Fig. S9E). Taken together, these results suggest that the *Med23* expression level is associated specifically with the prognoses of human lung cancer patients whose tumors contain activations in the Ras pathway. Therefore, *Med23* expression level could be used to predict the clinical outcomes of patients with Ras-active lung cancer.

To explore further the clinical prognostic value of MED23, we generated a therapeutic mouse model to test the possible clinical applications of MED23 manipulation. First, an A549 cell line with a doxycycline (Dox)-induced RNAi knockdown of *Med23* was established (Fig. 6C). The cells then were injected s.c. into nude mice to form a xenograft tumor. Mice with tumor volume of at least 50 mm³ were separated randomly into two groups. The treatment group was fed with water containing Dox (changed every 4 d) for 20 d to knock down *Med23* (Fig. 6D). The s.c. tumors of the Dox-treated animals were much smaller than those of the control group (without Dox treatment) (Fig. 6E). Significantly, and as is consistent with the relationship between *Med23* expression and clinical prognosis, the Dox-induced *Med23* RNAi significantly increased the life span of the xenografted mice ($P < 0.05$) (Fig. 6F). These data strongly support the idea that the attenuation of MED23 function is a restrictive force against Ras-mutated lung cancers. Therefore, MED23 may represent an attractive drug target for the clinical treatment of Ras-active lung cancers.

Discussion

In this work, we identified the Mediator subunit MED23 as an important regulator in lung cancer with oncogenic Ras signaling. By controlling a common set of cell-cycle- and cell-proliferation-related genes, MED23 selectively regulates the proliferative potential and the tumorigenicity of lung cancer cells with an oncogenic Ras mutation. The depletion of *Med23* specifically reduced the efficiency of transforming fibroblasts by Ras but not by c-Myc, underscoring the specific relationship between activated Ras and MED23 in oncogenesis. Furthermore, we observed that MED23 was up-regulated during Ras transformation and was overexpressed in the Ras-active lung cancer cell lines and in the lung tumors with hyperactive Ras activity. These data suggest that the elevation in MED23 expression could be caused by or result from Ras hyperactivity during lung carcinogenesis. Significantly, the expression level of *Med23* could be useful in predicting the clinical outcomes of patients with hyperactive Ras signatures, suggesting that the Mediator MED23 subunit may serve as a diagnostic marker for individualized therapy. Overall, our data suggest that MED23 is required for the Ras addiction during lung carcinogenesis.

Our study on the transcription factor ELK1 may help explain the specific relationship between Ras signaling and Mediator MED23 in lung tumorigenesis. Through specific interaction with MED23 upon Ras or MAPK activation, the transcription factor ELK1 controls the expression of a gene set that is specifically involved in the regulation of Ras-active cancer-cell proliferation and Ras transformation. Interestingly, the phosphorylation levels of ELK1 also are elevated in both lung cancer cell lines and clinical lung cancer samples with active Ras activities and in the process of Ras transformation are strongly correlated with the expression levels of MED23. In contrast, the low Ras activity indicated by the low levels of p-ELK1 and p-ERK were correlated with low MED23 expression in the lung cancer cell lines without activated Ras mutation; consequently, growth control of these cells is not MED23 dependent. Therefore, the selective requirement for MED23 in lung cancer might depend on the signal strength of the Ras–MAPK pathway, as indicated by the level of ELK1 phosphorylation. Thus, ELK1 phosphorylation might be responsible for relaying the mutual dependence between Ras hyperactivity and overexpressed MED23 in lung cancer.

Fig. 6. Expression of *Med23* is associated with the prognosis of patients who have Ras-active lung cancer and with the life span of the xenograft mouse model. (A and B) Predicting the prognosis of lung cancer patients using the expression level of *Med23*. Kaplan-Meier analysis was used to assess survival of lung cancer patients with either positive (A) or negative (B) Ras signatures as a function of high or low *Med23* expression (compared with the median). Log-rank *P* values indicate differences between the two groups. (C) Establishing an A549 cell line with inducible knockdown of *Med23*. Western blot analysis of lysates from the inducible *Med23*-RNAi A549 cells following Dox treatment; Ku86 and TBP were blotted as loading control. (D) Western blot analysis of the s.c. tumors formed by the inducible *Med23*-RNAi A549 cells following Dox treatment; Ku86 and β -actin were blotted as loading control. (E) Inducible *Med23*-RNAi A549 cells were injected s.c. and grown in nude mice with or without Dox treatment. Each data point is the mean volume of five or six primary tumors. **P* < 0.05, ***P* < 0.01; error bars indicate SD. (F) Kaplan-Meier survival curves are shown for the xenograft mice bearing tumors formed by inducible *Med23*-RNAi A549 cells with or without Dox treatment (*P* = 0.03).

Aberrant transcriptional regulation that results in malignant gene-expression patterns often accompanies carcinogenesis (39, 40). Many factors involved in transcriptional regulation are potential targets for cancer therapy, including p53 (41), HDAC (42), Myc (43), β -catenin (44), and elongation factor pTEFb (45), among others. Some of these targets are oncogenes that contribute directly to the malignant phenotype of certain types of tumors; others are nononcogenes that are important for maintaining or supporting the malignancy. The Mediator complex functions as an integrative hub for transmitting different signaling pathways through physical interactions between the various transcription factors and specific Mediator subunits. Recent studies (46–48), including this one, have highlighted the specificity of different Mediator subunits in regulating distinct types of cancers through controlling specific signaling and gene programs. The current study demonstrated that, by channeling hyperactive Ras signaling, the MED23 subunit plays a highly specific role in regulating Ras-driven lung cancer. The protein level of *Med23* was increased during Ras transformation and was up-regulated in lung cancer cell lines and in samples from patients who had lung cancer with hyperactive Ras activity. Because the *Med23* mRNA level also was increased during Ras transformation, we

examined the *Med23* mRNA levels in distinct lung cancer cell lines and clinical samples. However, we failed to detect consistent differences in the levels of *Med23* mRNA in normal and cancer tissues using a cDNA array and the Oncomine database (Fig. S10A and C). Moreover, no correlation with the Ras-mutation status in lung cancer cells lines was observed (Fig. S10B). These observations suggested that the elevated MED23 protein level in samples from cancer patients might be caused by the protein stabilization resulting from hyperactive Ras signaling. On the other hand, we also noticed that MED23 is not the only subunit that was up-regulated in the process of Ras transformation; indeed, most of the Mediator subunits examined were up-regulated during Ras transformation. The increase of the Mediator complex probably is required by the enhanced cell growth, although MED23 subunit still was specifically required for sensing the signal strength of the hyperactive Ras pathway.

To address further whether the MED23 is specifically important in Ras-driven tumorigenesis, we compared the roles of several other Mediator components in carcinogenesis. Although MED15 also was increased during Ras transformation, Med15 RNAi did not inhibit the efficiency of Ras transformation or the growth rate of lung cancer cells with Ras mutations. Also, unlike

MED23, MED15 protein levels did not correlate with the Ras activities in samples from lung cancer patients, and Med15 mRNA levels were not associated with the prognosis of patients with Ras-active lung cancer (Fig. S9). Med1 is another well-studied subunit of the Mediator complex which can act as a key regulator of the nuclear hormone receptor signaling through binding with Androgen Receptor or Estrogen Receptor and plays an important and specific coregulatory role in prostate cancer and breast cancer cell proliferation and survival (46). Moreover, the Mediator kinase subunit CDK8, which is important for the aberrant activation of the canonical WNT/ β -catenin pathway, has been associated with a substantial percentage of colorectal cancers (47). Most recently, Med12 was found to be mutated in a large percentage of uterine leiomyomas and might contribute to cancer progression (48). These studies of other Mediator subunits in different types of cancer further highlight the specificity of MED23 in Ras-driven tumorigenesis.

Although *Ras* gene family members are mutated in ~30% of human tumors, efforts to develop Ras-directed molecular therapeutics have proven challenging thus far (5, 6). An alternative approach is to search for inhibitors that bypass Ras to target the various Ras downstream signaling effectors, such as B-raf and MEK (10, 12). Many new therapeutic agents currently are in clinical trials, and some of them have been shown to be efficacious in treating tumors with Ras mutations (49, 50). However, resistance to these targeted therapies often occurs because the secondary mutations of the kinase abrogate drug binding or because other mutations occur along the pathway and reactivate the oncogenic signaling (51, 52). MED23, which functions as a nuclear target of the Ras-MAPK kinase cascade and specifically controls a small set of genes, could be a target for drugs directed against aberrant Ras signal transduction and might be helpful in overcoming the prevalence of drug resistance. Because all the mutated oncogenes in Ras-MAPK pathway, such as EGFR, Ras, and Raf, share a common signal-transduction cascade, patients with mutations in any of these oncogenes might benefit from targeting MED23. Indeed, we observed that the loss of MED23 also could inhibit the growth of cancer cells with EGFR or B-raf mutations (Fig. S4 C and D). Tumors often depend on the sustained expression and activity of a single aberrant initiating oncogene for the maintenance of the malignant phenotype and better cell survival (53). This phenomenon, called “oncogene addiction,” provides a rationale for molecular targeted therapy and offers potential therapeutic opportunities (54, 55). Our study revealed that cancer cells harboring K-Ras mutations not only are addicted to oncogenic K-Ras but also rely on the entire Ras-MAPK pathway, including the downstream cofactor MED23, to maintain the addiction, suggesting that targeting MED23 might be used alone or in combination with other therapeutic agents to counteract the addictive Ras pathway. MED23, ELK1, or their interaction interface may represent multiple pharmaceutical targets in patients with Ras-mutated lung cancer, for whom currently there is no effective therapy.

Experimental Procedures

Plasmids and Materials. The *Med23*, *Elk1*, and *Med15* RNAi sequences were determined using the Dharmacon siRNA designing tool and were cloned into pSiren-RetroQ (Clontech) (see [SI Experimental Procedures](#) for oligonucleotide sequences). pMXs-hc-MYC was obtained from Addgene. pWz1-Ha-RasV12 was a generous gift from Peiqing Sun (The Scripps Research Institute, San Diego). pLVUT-tR-KRAB was a generous gift from Guoliang-Xu (Institute of Biochemistry and Cell Biology, Shanghai). Doxycycline used to induce *Med23* RNAi was obtained from BD Pharmingen. Cisplatin was from obtained Sigma-Aldrich. Human lung cancer samples were purchased from Shanghai Biochip Company Ltd. All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Shanghai Institutes for Biological Sciences.

Cell Culture. A549, H1299, CRL-5807, HTB-177, H2122, HTB-57, H522, HTB-182, and CRL-5889 cells were obtained from Hongbin Ji (Institute of Biochemistry

and Cell Biology, Shanghai). CRL-2078 and CRL-2741 were from the American Type Culture Collection. WI-38, MRC-5, PANC-1, DU145, H1650, and A375 cells were from the Type Culture Collection of the Chinese Academy of Sciences. *Med23*^{+/+} and *Med23*^{-/-} MEFs were isolated from 9.5-d embryos and self-immortalized following a standard protocol (30). H1299, CRL-5807, HTB-177, H2122, H522, HTB-182, CRL-5889, and H1650 cells were grown in RPMI 1640 (HyClone) containing 10% (vol/vol) FBS (HyClone). A549, PANC-1, DU145, A375, and immortalized MEFs were cultured in DMEM containing 10% (vol/vol) FBS. HTB-57, WI-38, and MRC-5 cells were maintained in Minimum Essential Medium (Hyclone) containing 10% (vol/vol) FBS. CRL-2078 and CRL-2741 cells were cultured in keratinocyte serum-free medium (Gibco) with 5 ng/mL human recombinant EGF and 0.05 mg/mL bovine pituitary extract. All cells were maintained inside the incubator with 5% CO₂.

Western Blot Analysis and Real-Time PCR. Methods for real-time PCR and Western blot analysis were described previously (21, 30). Antibodies for Western blot were as follows: MED23 (BD), MED1 (Santa Cruz), MED15 (Abcam), H-Ras (Santa Cruz), c-Myc (Santa Cruz), ELK1 (Santa Cruz), Ku86 (Santa Cruz), TBP (Santa Cruz), β -actin (Sigma-Aldrich), caspase3 (Santa Cruz), cleaved caspase3 (Cell Signaling), ERK (Santa Cruz), p-ERK (Santa Cruz), AKT (Cell Signaling), and p-AKT (Cell Signaling). The MED16 antibody was a generous gift from Robert Roeder (The Rockefeller University, New York). See [SI Experimental Procedures](#) for the primer sequences used for real-time PCR analysis.

Retrovirus Infection. Stable cell lines to knock down or overexpress a gene of interest were established following the manufacturer's recommendations (Clontech) and have been described previously (21, 30). Retroviruses were generated following the cotransfection of recombinant pSiren-RetroQ plasmids with pCL10A1 helper plasmid into 293T cells using Lipofectamine 2000 (Invitrogen). Tissue-culture supernatants containing retroviruses were harvested 48 h posttransfection and passed through a 0.45- μ m filter. Cells were plated into six-well plates before retroviral infection. Virus-containing supernatants were supplemented with 20 μ g/mL Polybrene (Sigma-Aldrich) and added to the cells for spin infection by centrifugation at 1,258 $\times$ g at 30 °C for 1.5 h. Twenty-four hours after spin infection, MEFs were selected with 50 μ g/mL puromycin or 500 μ g/mL hygromycin (Sigma-Aldrich). A549 cells and the other lung cancer cells were selected with 5 μ g/mL puromycin.

Cell-Proliferation Assay. After puromycin selection, si-Ctrl and si-Med23 cells were trypsinized and were seeded at 2×10^4 cells per well in 12-well plates. Cells were allowed to proliferate for an additional 6 d with medium changed after 3 d. Cell number was measured every day or 6 d after plating by a CYTORECON cell counter (GE Healthcare). Experiments were done in triplicate.

Growth Assays in Soft Agar. Soft agar growth assays were performed as described. For anchorage-independent growth, 2×10^4 A549 cells or transfected MEFs were resuspended in a medium containing 0.3% low-melting-point agarose and were plated onto a solidified bottom layer medium containing 0.5% agarose in six-well plates. The colonies that formed at 2 wk were stained with 1 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 6 h and were imaged.

Tumor-Formation Assays in Athymic Nude Mice. Five million A549 cells or one million transformed MEFs were injected s.c. into the flanks of 6-wk-old female nude mice in 100 μ L of serum-free DMEM. Tumor growth was monitored for 7–10 wk for A549 cells and for 4–8 wk for transformed MEFs. The tumors were measured with a caliper every 4 d, and tumor volume was calculated as $0.5 \times L \times W \times W$. The tumors were weighed, processed, and imaged after surgical removal.

Microarray Analysis. The global expression of control, si-Med23, or si-Elk1 A549 cells was identified using Affymetrix Human Genome U133 plus 2.0 Arrays, and the data were extracted using RMA implemented in R. Present/absent calls were generated by MAS5 in R for human genome U133 plus 2.0 arrays; *t* tests were performed on predefined sample groups with $n = 3$. The log₂ fold-change was generated from control subtracting knockdown. The heat map was generated using z-scores across all the samples. The GO annotations were performed using GOMiner (<http://discover.nci.nih.gov/gominer/index.jsp>).

Tissue Microarray and Immunohistochemistry. Three tissue microarray (TMA) chips containing a total of 188 pairs of tumors and matched adjacent tissues were obtained from Shanghai Biochip Company Ltd. Immunohistochemistry of the TMA Chips was carried out using primary antibodies against MED23 (BD), MED15 (Abcam), p-ERK (Santa Cruz), and p-ELK1 (Santa Cruz). Briefly, sections

were dewaxed, hydrated, and washed. After neutralization of endogenous peroxidase and microwave antigen retrieval, slides were preincubated with blocking serum and then were incubated overnight with each antibody. Subsequently, the sections were serially rinsed, incubated with second antibodies, and treated with HRP-conjugated streptavidin. Reaction products were visualized with 3, 3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. For each antibody, all tissue microarray staining was done in a single experiment, including negative staining controls.

- Jemal A, et al. (2009) Cancer statistics, 2009. *CA Cancer J Clin* 59:225–249.
- Wistuba II, Gazdar AF (2006) Lung cancer preneoplasia. *Annu Rev Pathol* 1:331–348.
- Herbst RS, Heymach JV, Lippman SM (2008) Lung cancer. *N Engl J Med* 359:1367–1380.
- Karnoub AE, Weinberg RA (2008) Ras oncogenes: Split personalities. *Nat Rev Mol Cell Biol* 9:517–531.
- Schubert S, Shannon K, Bollag G (2007) Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* 7:295–308.
- Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG (2007) Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* 6:541–555.
- Cox AD, Der CJ (2002) Ras family signaling: Therapeutic targeting. *Cancer Biol Ther* 1: 599–606.
- Downward J (2003) Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3:11–22.
- Riely GJ, Marks J, Pao W (2009) KRAS mutations in non-small cell lung cancer. *Proc Am Thorac Soc* 6:201–205.
- Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 26:3291–3310.
- Engelman JA, et al. (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351–1356.
- Sebolt-Leopold JS, Herrera R (2004) Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nat Rev Cancer* 4:937–947.
- Stadler WM (2005) Targeted agents for the treatment of advanced renal cell carcinoma. *Cancer* 104:2323–2333.
- Kornberg RD (2005) Mediator and the mechanism of transcriptional activation. *Trends Biochem Sci* 30:235–239.
- Taatjes DJ (2010) The human Mediator complex: A versatile, genome-wide regulator of transcription. *Trends Biochem Sci* 35:315–322.
- Malik S, Roeder RG (2010) The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nat Rev Genet* 11:761–772.
- Kang YK, Guermah M, Yuan CX, Roeder RG (2002) The TRAP/Mediator coactivator complex interacts directly with estrogen receptors alpha and beta through the TRAP220 subunit and directly enhances estrogen receptor function in vitro. *Proc Natl Acad Sci USA* 99:2642–2647.
- Kato Y, Habas R, Katsuyama Y, Näär AM, He X (2002) A component of the ARC/Mediator complex required for TGF beta/Nodal signalling. *Nature* 418:641–646.
- Kim S, Xu X, Hecht A, Boyer TG (2006) Mediator is a transducer of Wnt/beta-catenin signaling. *J Biol Chem* 281:14066–14075.
- Stevens JL, et al. (2002) Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. *Science* 296:755–758.
- Wang G, et al. (2005) Mediator requirement for both recruitment and postrecruitment steps in transcription initiation. *Mol Cell* 17:683–694.
- Singh N, Han M (1995) sur-2, a novel gene, functions late in the let-60 ras-mediated signaling pathway during *Caenorhabditis elegans* vulval induction. *Genes Dev* 9: 2251–2265.
- Scholl C, et al. (2009) Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. *Cell* 137:821–834.
- Barbie DA, et al. (2009) Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 462:108–112.
- Ferbeyre G (2007) Barriers to Ras transformation. *Nat Cell Biol* 9:483–485.
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88:593–602.
- Kamijo T, et al. (1997) Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 91:649–659.
- Sage J, et al. (2000) Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. *Genes Dev* 14:3037–3050.
- Buchwalter G, Gross C, Wasylyk B (2004) Ets ternary complex transcription factors. *Gene* 324:1–14.
- Wang W, et al. (2009) Mediator MED23 links insulin signaling to the adipogenesis transcription cascade. *Dev Cell* 16:764–771.
- Cantin GT, Stevens JL, Berk AJ (2003) Activation domain-mediator interactions promote transcription preinitiation complex assembly on promoter DNA. *Proc Natl Acad Sci USA* 100:12003–12008.
- Boros J, et al. (2009) Elucidation of the ELK1 target gene network reveals a role in the coordinate regulation of core components of the gene regulation machinery. *Genome Res* 19:1963–1973.
- Vijayvargia R, May MS, Fondell JD (2007) A coregulatory role for the mediator complex in prostate cancer cell proliferation and gene expression. *Cancer Res* 67: 4034–4041.
- Land H, Parada LF, Weinberg RA (1983) Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304:596–602.
- Sun P, et al. (2007) PRK is essential for ras-induced senescence and tumor suppression. *Cell* 128:295–308.
- Ruley HE (1983) Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature* 304:602–606.
- Bhattacharjee A, et al. (2001) Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc Natl Acad Sci USA* 98:13790–13795.
- Luo J, et al. (2009) A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell* 137:835–848.
- Darnell JE, Jr. (2002) Transcription factors as targets for cancer therapy. *Nat Rev Cancer* 2:740–749.
- Konstantinopoulos PA, Papavassiliou AG (2011) Seeing the future of cancer-associated transcription factor drug targets. *JAMA* 305:2349–2350.
- Selivanova G, Wiman KG (2007) Reactivation of mutant p53: Molecular mechanisms and therapeutic potential. *Oncogene* 26:2243–2254.
- Richon VM, O'Brien JP (2002) Histone deacetylase inhibitors: A new class of potential therapeutic agents for cancer treatment. *Clin Cancer Res* 8:662–664.
- Pelengaris S, Khan M, Evan GI (2002) Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* 109:321–334.
- Verma UN, Surabhi RM, Schmalstieg A, Becerra C, Gaynor RB (2003) Small interfering RNAs directed against beta-catenin inhibit the in vitro and in vivo growth of colon cancer cells. *Clin Cancer Res* 9:1291–1300.
- Navone NM (2010) P-TEFb joins the family of cdks in oncology, promotes cell growth of cancer cells. *Cell Cycle* 9:2935.
- Spaeth JM, Kim NH, Boyer TG (2011) Mediator and human disease. *Semin Cell Dev Biol* 22:776–787.
- Firestein R, et al. (2008) CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. *Nature* 455:547–551.
- Mäkinen N, et al. (2011) MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. *Science* 334:252–255.
- Adjei AA, et al. (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. *J Clin Oncol* 26:2139–2146.
- Bollag G, et al. (2010) Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 467:596–599.
- Puzanov I, Burnett P, Flaherty KT (2011) Biological challenges of BRAF inhibitor therapy. *Mol Oncol* 5:116–123.
- Little AS, et al. (2011) Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. *Sci Signal* 4:ra17.
- Sharma SV, Settleman J (2007) Oncogene addiction: Setting the stage for molecularly targeted cancer therapy. *Genes Dev* 21:3214–3231.
- Weinstein IB, Joe AK (2006) Mechanisms of disease: Oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 3:448–457.
- Weinstein IB, Joe A (2008) Oncogene addiction. *Cancer Res* 68:3077–3080, discussion 3080.