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SHORT COMMUNICATION

Foxm1 transcription factor is required for the initiation of lung tumorigenesis by oncogenic Kras^{G12D}

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Lung cancer is the leading cause of deaths in cancer patients in the United States. Identification of new molecular targets is clearly needed to improve therapeutic outcomes of this devastating human disease. Activating mutations in K-Ras oncogene and increased expression of FOXM1 protein are associated with poor prognosis in patients with non-small-cell lung cancer. Transgenic expression of activated Kras^{G12D} in mouse respiratory epithelium is sufficient to induce lung adenocarcinomas; however, transcriptional mechanisms regulated by K-Ras during the initiation of lung cancer remain poorly understood. Foxm1 transcription factor, a downstream target of K-Ras, stimulates cellular proliferation during embryogenesis, organ repair and tumor growth, but its role in tumor initiation is unknown. In the present study, we used transgenic mice expressing Kras^{G12D} under control of *Sftpc* promoter to demonstrate that Foxm1 was induced in type II epithelial cells before the formation of lung tumors. Conditional deletion of *Foxm1* from Kras^{G12D}-expressing respiratory epithelium prevented the initiation of lung tumors *in vivo*. The loss of *Foxm1* inhibited expression of K-Ras target genes critical for the nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) pathways, including *Ikbkb, Nfkb1, Nfkb2, Rela, Jnk1, N-Myc, Pttg1* and *Cdkn2a*. Transgenic overexpression of activated FOXM1 mutant was sufficient to induce expression of these genes in alveolar type II cells. FOXM1 directly bound to promoter regions of *Ikbkb, Nfkb2, N-Myc, Pttg1* and *Cdkn2a*, indicating that these genes are direct FOXM1 targets. FOXM1 is required for K-Ras-mediated lung tumorigenesis by activating genes critical for the NF-κB and JNK pathways.

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INTRODUCTION

Lung cancer is the leading cause of deaths in cancer patients worldwide with the current 5-year survival of 8–12%. Existing treatments for lung cancers have not significantly improved patient survival, leading to a critical need for new therapeutic approaches.[1] Lung adenocarcinoma, the most common type of non-small-cell lung cancer (NSCLC), exhibits metastases before clinical symptoms become apparent, thus reducing successful treatment options.[2] NSCLC arise from pulmonary epithelial cells and are often associated with inactivation of tumor-suppressor genes and activating mutations in K-Ras oncogene. Identification of new molecular targets is clearly needed to improve therapeutic outcomes of this devastating human disease.

Expression levels of FOXM1 protein (also known as HFH-11B, Trident, Win or MPP2) are dramatically increased in NSCLC cancers from mice and humans.[3] FOXM1 is a nuclear protein from the Forkhead Box (FOX) family of transcription factors. FOXM1 stimulates cellular proliferation during embryogenesis, organ repair and tumor growth by activating transcription of multiple cell cycle regulatory genes, including Cdc25A, cyclins B1 and A2, Cdc25B, Polo-like and Aurora B kinases.[4,5] FOXM1 is induced before the onset of cellular proliferation and deletion of the *Foxm1* gene in transgenic mice inhibits the progression of quiescent cells into the cell cycle (ref). Positive correlation was shown between increased FOXM1 and poor prognosis in NSCLC patients.[6] In mice, overexpression of FOXM1 in *Rosa26-FOXM1* transgenic mice accelerated proliferation of tumor cells and increased the number and size of lung tumors after treatment with 3-methylcholanthrene/butylated hydroxytoluene.[7] Likewise, deletion of *Foxm1* caused a significant reduction in numbers and sizes of lung adenomas induced by either 3-methylcholanthrene/butylated hydroxytoluene or urethane.[3,8] Activated ERK and p38, both of which are known targets of the Kras signaling pathway, directly activate the Foxm1b protein in cultured tumor cells and *in vivo* (ref).

Although these studies demonstrated a critical role of FOXM1 in proliferation of neoplastic cells during lung tumor growth, the role of FOXM1 in tumor initiation is unknown. In the present study, we used transgenic mice expressing Kras^{G12D} under control of *Sftpc* promoter to examine the kinetic of Foxm1 expression after activation of K-Ras/Raf/ERK signaling pathway *in vivo*. We demonstrated that Foxm1 is induced in alveolar type II cells before the formation of lung tumors. Conditional deletion of *Foxm1* from type II cells prevented the initiation of lung tumors by oncogenic K-Ras^{G12D} through inhibition of genes critical for the nuclear factor-κB (NF-κB) and JNK pathways.

RESULTS AND DISCUSSION

Kras^{G12D} induces Foxm1 protein in lung tumor cells and alveolar type II cells

Previous studies demonstrated that transgenic overexpression of activated Kras^{G12D} in mouse respiratory epithelium is sufficient to induce lung adenocarcinomas.[9–11] Oncogenic FOXM1 protein is a

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downstream target of Ras/ERK and Ras/p38 pathways in cultured tumor cells and mouse embryos[12–15] but its role in K-Ras-mediated lung carcinogenesis is unknown. To examine Foxm1 expression during K-Ras-mediated lung tumorigenesis *in vivo*, transgenic mice that express a mutant $Kras^{G12D}$ transcript under control of *Sftpc* promoter (SPC-rtTA/TetO-$Kras^{G12D}$ mice,[9]) were used. Consistent with previous studies,[9] doxycycline (Dox)-mediated activation of $Kras^{G12D}$ transgene induced lung adenocarcinomas in a time-dependent manner (Figure 1a). Activation of $Kras^{G12D}$-induced phosphorylated extracellular signal-regulated kinase (pERK) and Foxm1 proteins in lung tumor and alveolar regions (Figure 1a). Neither pERK nor Foxm1 were found in lungs of mice without Dox (Figure 1a). All lung tumors in SPC-rtTA/TetO-$Kras^{G12D}$ mice contained proSP-C (Supplementary Figure 1), a specific marker for type II alveolar epithelial cells,[16] indicating that these tumors were derived from a distal epithelial lineage. Interestingly, both pERK and Foxm1 were increased in type II cells before the formation of lung tumors (Figures 1b and c), implicating Foxm1 in tumor initiation.

Conditional deletion of *Foxm1* in respiratory epithelial cells prevents K-Ras-mediated lung tumorigenesis

To determine whether Foxm1 is required for $Kras^{G12D}$-driven lung tumorigenesis, we generated quadruple transgenic mice containing SPC-rtTA, TetO-$Kras^{G12D}$ and TetO-Cre transgenes as well as

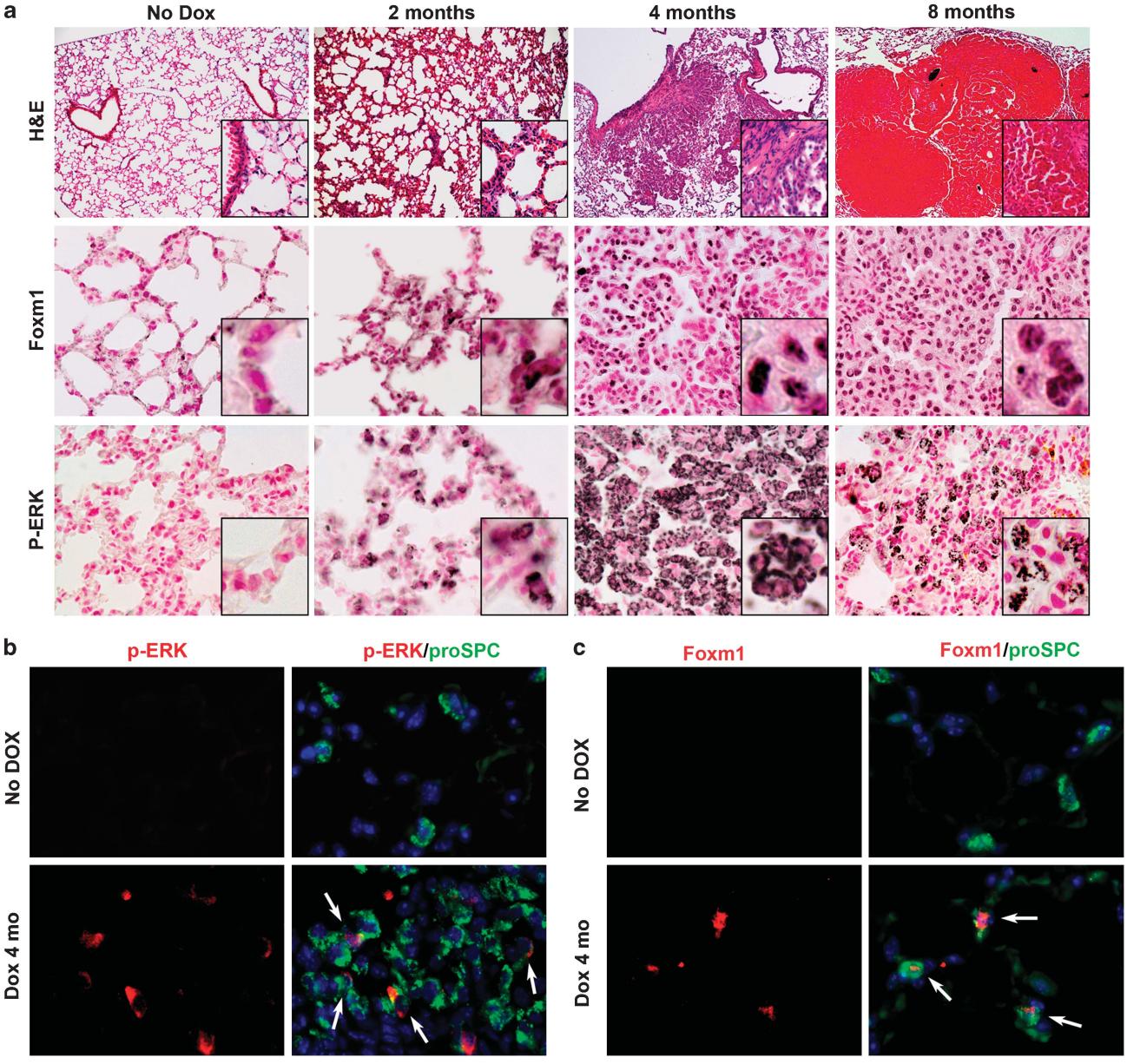

Figure 1. Foxm1 and pERK are expressed in alveolar type II cells and tumor cells after activation of $Kras^{G12D}$. (**a**) Lungs from SPC-rtTA/TetO-$Kras^{G12D}$ mice that were given Dox for 2, 4 or 8 months (mo), were paraffin-embedded, sectioned and stained with either hematoxylin and eosin (H&E) or antibodies against Foxm1 or pERK (brown). Lung sections were counterstained with nuclear fast red (red nuclei). Activation of $Kras^{G12D}$-induced lung adenocarcinomas in a time-dependent manner. Increased staining for pERK and Foxm1 was observed in alveolar regions and lung tumors after Dox treatment. pERK and Foxm1 were absent in lungs of mice without Dox (left panels). Insets show high magnification of Foxm1-positive and pERK-positive cells. (**b**, **c**) proSP-C colocalizes with p-ERK (**b**) and Foxm1 (**c**) in alveolar type II cells. Neither pERK nor Foxm1 were found in type II cells in the absence of Dox. Cell nuclei were counterstained with 4′-6-diamidino-2-phenylindole. Magnifications: $\times$50, top panels; $\times$400, remaining panels in **a**; $\times$1000, **b**, **c**.

the *Foxm1*-floxed allele (*SPC–rtTA*$^{tg/-}$/*TetO-Kras*$^{tg/-}$/*TetO-Cre*$^{tg/-}$/*Foxm1*$^{fl/fl}$ or epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice). In these mice, Dox induces simultaneous expression of Kras^{G12D} and Cre proteins in alveolar type II cells and airway Clara cells, resulting in Cre-mediated excision of exons 4–7 of the *Foxm1* gene that encode DNA binding and transcriptional activation domains of the Foxm1 protein (Figure 2a). Efficient deletion of the *Foxm1-floxed* allele by the *SPC–Cre* transgene was shown in our previous studies.[8,16] Mice without Cre (*SPC–rtTA*$^{tg/-}$/*TetO-Kras*$^{tg/-}$/*Foxm1*$^{fl/fl}$ or epKras^{G12D}/*Foxm1*$^{fl/fl}$ mice) were used as controls. After Dox treatment, microCT scan imaging and computer-based three-dimensional reconstruction of whole mouse lungs were performed to determine the number and volume of lung tumors. Activation of Kras^{G12D}-induced lung adenocarcinomas in control epKras^{G12D}/*Foxm1*$^{fl/fl}$ mice (Figure 2b), which was confirmed by histological evaluation of these tumors (Figure 2c). Increased expression of pERK and pAKT was observed in tumor cells (Supplementary Figure 3) and was consistent with activation Raf/ERK and PI3K/AKT signaling pathways in Kras^{G12D}-induced tumors.[17,18] In contrast, deletion of *Foxm1* from Kras^{G12D}-expressing respiratory epithelium dramatically reduced the number and size of lung tumors (Figures 2b–d). Although approximately 25% of Kras/*Foxm1*$^{-/-}$ mice still developed single lung tumors (Figure 2d), these tumors were positive for Foxm1 after histological examination (data not shown), indicating that they developed because of incomplete deletion of *Foxm1*.

Interestingly, deletion of *Foxm1* did not protect from Kras^{G12D}-mediated hyperplasia in airway epithelium and terminal bronchioles of Dox-treated epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice (Figure 2c and Supplementary Figure 2). pERK and pAKT were detected in

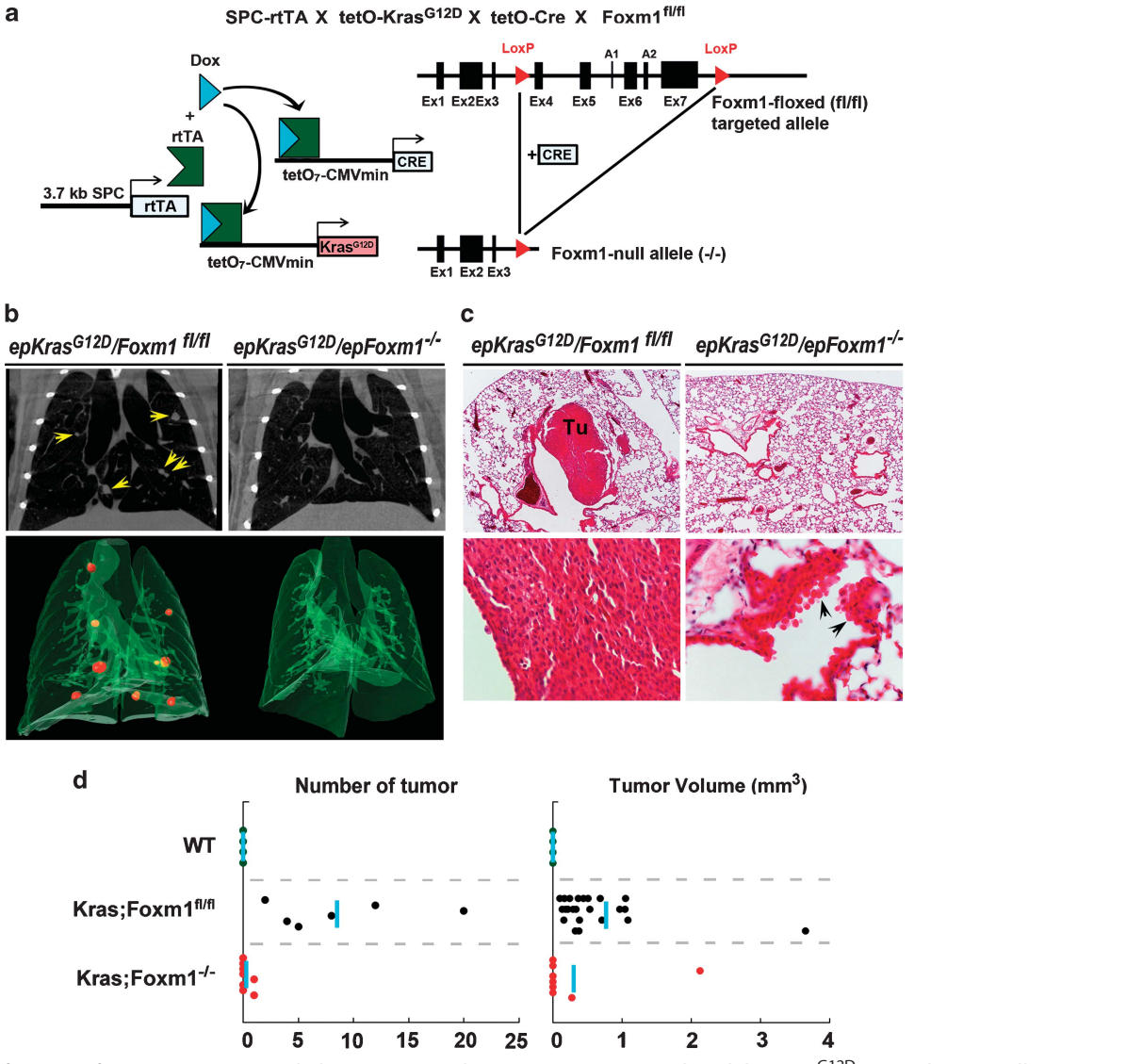

Figure 2. Deletion of Foxm1 from respiratory epithelium prevents lung tumorigenesis induced by Kras^{G12D}. (**a**) Schematic illustrates conditional activation of *Kras*^{G12D} transgene and deletion of *Foxm1*$^{fl/fl}$ alleles in lung epithelium using Tet-On system. After Dox treatment, Cre deletes exons 4–7 of the *Foxm1* gene that encode DNA binding and transcriptional activation domains of the Foxm1 protein. (**b**, **c**) Deletion of *Foxm1* prevents Kras^{G12D}-mediated lung tumorigenesis. Transgenic mice were treated with Dox for 8 months. Lung tumors are shown with arrows in microCT images and with red color in computer-based three-dimensional reconstruction of whole mouse lungs (**b**). Hematoxylin and eosin staining of paraffin-embedded lung sections (**c**) shows lung tumors (Tu) in Dox-treated *SPC–rtTA/TetO-Kras*^{G12D}/*Foxm1*$^{fl/fl}$ mice (epKras^{G12D}/*Foxm1*$^{fl/fl}$) but not in Dox-treated *SPC–rtTA/TetO-Kras*^{G12D}/*TetO-Cre*/*Foxm1*$^{fl/fl}$ mice (epKras^{G12D}/ep*Foxm1*$^{-/-}$). Epithelial hyperplasia is indicated by black arrows in epKras^{G12D}/ep*Foxm1*$^{-/-}$ lungs (**c**). (**d**) The number of lung tumors and tumor volumes were determined from microCT images of epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice ($n=8$), epKras^{G12D}/*Foxm1*$^{fl/fl}$ mice ($n=6$) and wild-type (WT) mice ($n=4$). Magnifications: $\times$50, top panels in **c**; $\times$400, bottom panels in **c**.

hyperplastic respiratory epithelium of epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice (Supplementary Figure 3), a finding consistent with increased K-Ras signaling. Proliferating epithelial cells were found in Foxm1-deficient epithelium as demonstrated by immunostaining for proliferation-specific markers Ki-67 and phosphorylated histone H3 (Supplementary Figure 4). Thus, deletion of *Foxm1* does not prevent cellular proliferation in hyperplastic epithelial cells. Altogether, our results suggest that Foxm1 is required for progression of epithelial hyperplasia into the lung tumor by acting downstream of the K-Ras/pERK signaling pathway.

Deletion of *Foxm1* prevents K-Ras-mediated activation of genes critical for the JNK and NF-κB signaling pathways

To determine Foxm1 requirements in oncogenic K-Ras signaling, we focused on early events of tumor initiation. epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice were treated with Dox for 7 days to activate Kras^{G12D} and Cre in the adult lung (Figure 3a). Dox-treated epKras^{G12D}/*Foxm1*$^{fl/fl}$ mice and mice without Dox were used as controls. This short Dox exposure induced pERK, pAKT and Foxm1 proteins in type II cells, but was insufficient to induce epithelial hyperplasia or lung tumors in all groups of mice (data not shown).

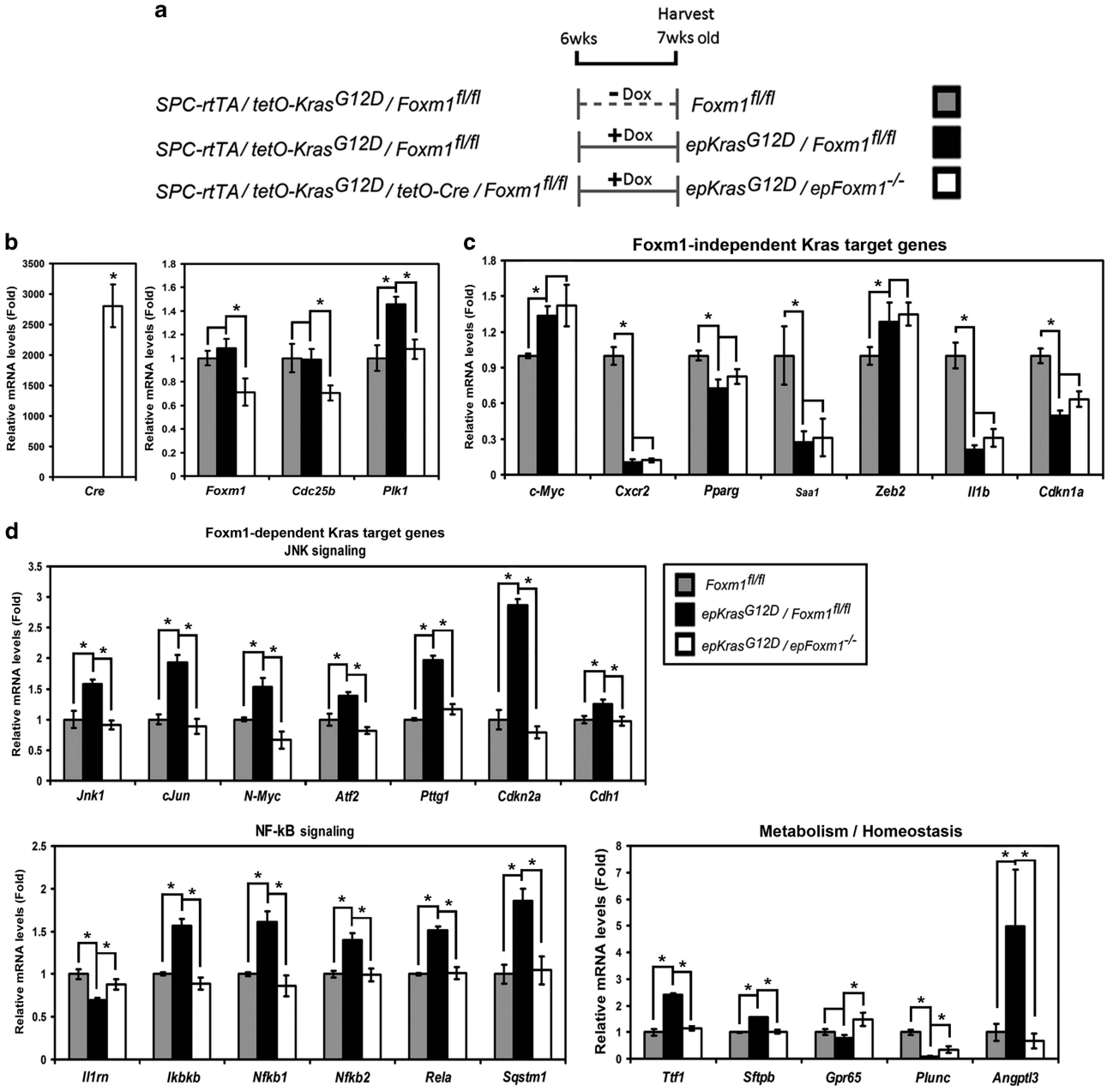

Figure 3. Deletion of Foxm1 influences mRNA levels of K-Ras target genes. (**a**) Experimental design of Foxm1 deletion in Kras^{G12D}-expressing respiratory epithelium. Experimental epKras^{G12D}/ep*Foxm1*$^{-/-}$ and control epKras^{G12D}/*Foxm1*$^{fl/fl}$ mice were given Dox for 7 days to induce Kras^{G12D} and Cre transgenes. Additional controls included *Foxm1*$^{fl/fl}$ mice without Dox. Four mice were used in each group. (**b**–**d**) Quantitative reverse transcription PCR was used to examine expression of K-Ras target genes in whole lung RNA. Deletion of *Foxm1* influenced expression of K-Ras targets genes associated with JNK pathway, NF-κB pathway and homeostasis/metabolism (**d**). K-Ras target genes that are independent of Foxm1 are shown in **c**. mRNA levels were normalized to β-actin mRNA. Data represent mean ± s.d. A *P* value <0.05 is shown with asterisk ($n=4$ mice per group).

Dox-treated epKras^{G12D}/ep*Foxm1*$^{-/-}$ lungs displayed an increase in Cre mRNA and a decrease in mRNAs of Foxm1 and its target genes, *Cdc25b* and *Plk1* (Figure 3b), findings consistent with deletion of *Foxm1* by the Cre recombinase. To identify K-Ras target genes that are dependent on Foxm1, expression of 25 downstream targets of K-Ras[17,18] was examined by real-time reverse transcription PCR. Consistent with previous studies,[17,19–22] activation of K-Ras decreased mRNA levels of Cxcr2, IL1b, Saa1, Pparg and Cdkn1a, whereas c-Myc and Zeb2 mRNAs were increased (Figure 3c). Deletion of *Foxm1* did not influence mRNA levels of these genes in epKras^{G12D}/ep*Foxm1*$^{-/-}$ lungs (Figure 3c), indicating that expression of these K-Ras targets is independent of Foxm1. In contrast, deletion of *Foxm1* prevented K-Ras-mediated changes in expression of *Ttf1*, *Sftpb*, *Gpr65*, *Plunc* and *Angptl3* (Figure 3d), all of which are critical for proper homeostasis, metabolism and function of lung epithelial and tumor cells.[23–25] Mutations in *TTF1* were found in patients with squamous cell carcinoma,[26] whereas mutations in *Sftpb* are associated with chronic interstitial lung disease.[27] *Gpr65*, *Plunc* and *Angptl3* were implicated in tumor growth and lipid metabolism.[24,25,28] Thus, Foxm1 regulates a subset of K-Ras target genes during tumor initiation.

Consistent with previous studies,[29,30] activation of Kras^{G12D} increased mRNA levels of genes critical for JNK signaling (Figure 3d). Deletion of *Foxm1* prevented upregulation of JNK1 in epKras^{G12D}/ep*Foxm1*$^{-/-}$ lungs (Figure 3d), a result consistent with transcriptional regulation of the *Jnk1* promoter by Foxm1.[31] mRNAs of JNK1 target genes, including *cJun*, *N-Myc*, *Atf2*, *Pttg1*, *Cdkn2a* and *Cdh1*, were decreased in Foxm1-deficient lungs (Figure 3d), indicating decreased JNK1 signaling. Furthermore, Kras^{G12D} increased mRNA levels of genes critical for NF-κB signaling (Figure 3d), a finding consistent with activation of cell cycle. Deletion of *Foxm1* prevented K-Ras-mediated changes in expression of NF-κB-associated genes, such as *Ikbkb*, *Nfkb1*, *Nfkb2*, *Rela*, *IL1rn* and *Sqstm1* (Figure 3d). As both JNK and NF-κB pathways are required for the initiation of lung tumorigenesis by activated Kras^{G12D} *in vivo*,[30,32] inhibition of these signaling pathways may contribute to tumor resistance in epKras^{G12D}/ep*Foxm1*$^{-/-}$ mice. Altogether, our data demonstrate that initiation of lung tumorigenesis by oncogenic Kras^{G12D} requires the Foxm1 transcription factor, which activates genes critical for the JNK and NF-κB signaling pathways.

Overexpression of FOXM1 in alveolar type II cells increases expression of genes critical for the JNK and NF-κB signaling pathways

To determine whether FOXM1 is sufficient to induce genes associated with the JNK and NF-κB pathways, we used double

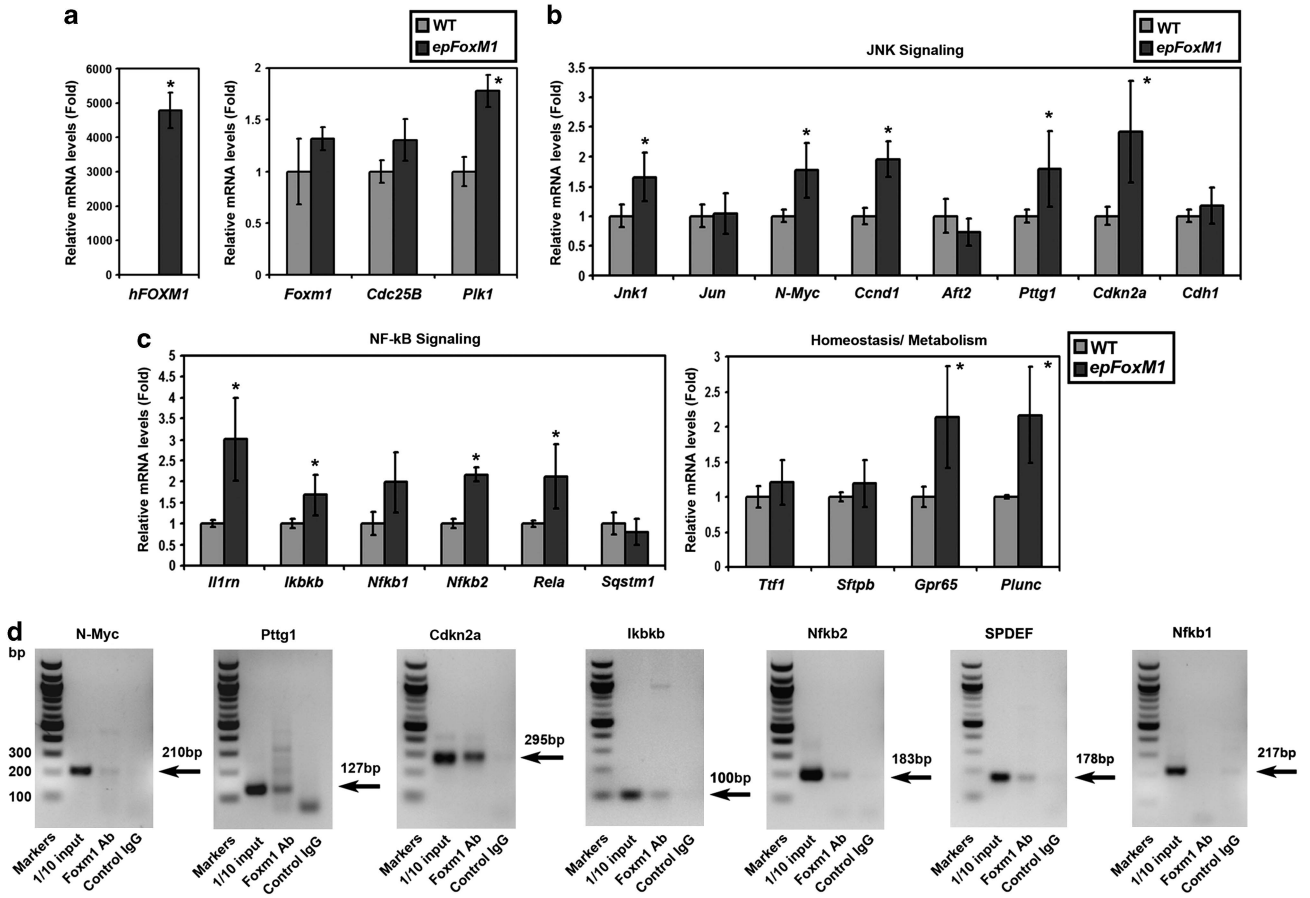

Figure 4. Expression of activated hFOXM1 mutant in type II cells increases mRNA levels of genes associated with JNK pathway and NF-κB pathway. SPC-rtTA/TetO-hFOXM1 and control wild-type (WT) mice ($n = 4$ mice per group) were treated with Dox from postnatal day 3 (P3) to P30. Lungs were used for purification of alveolar type II cells. (**a**–**c**) Quantitative reverse transcription PCR was used to examine mRNA levels of K-Ras target genes in purified type II cells. Expression of activated FOXM1 mutant (hFOXM1) was increased in type II cells from Dox-treated SP-C-rtTA/TetO-hFOXM1 mice (**a**). hFOXM1 increased mRNA levels of Plk1 (**a**), as well as genes associated with JNK pathway (**b**), NF-κB pathway and homeostasis/metabolism (**c**). mRNAs of endogenous (mouse) Foxm1 and Cdc25B were not changed. mRNA levels were normalized to β-actin mRNA. Data represent mean ± s.d. A P value < 0.05 is shown with asterisk. (**d**) Chromatin immunoprecipitation assay was performed in cultured human lung epithelial BEAS-2B cells. Endogenous FOXM1 protein specifically bound to the promoter regions of *N-Myc*, *Pttg1*, *Cdkn2a*, *Ikbkb* and *Nfkb2* genes. FOXM1 did not bind to the Nfkb1 promoter region. FOXM1 target gene *Spdef* was used as positive control.

transgenic mice expressing an activated human FOXM1 mutant protein (hFOXM1) under control of Dox-inducible *Sftpc* promoter (SPC-rtTA/TetO-hFOXM1 mice[33]). After activation of hFOXM1, alveolar type II cells were purified from transgenic lungs and used to prepare total RNA. Type II cells from Dox-treated TetO-hFOXM1 mice were used as controls. The purity of type II cell populations was 90–95% (data not shown). Transgenic hFOXM1 mRNA was induced in type II cells from SPC-rtTA/TetO-hFOXM1 mice but was absent in controls (Figure 4a), indicating an efficient activation of the hFOXM1 transgene. Plk1 mRNA was increased, whereas mRNAs of endogenous Foxm1 and Cdc25B were not changed (Figure 4a). Consistent with the loss-of-function studies (Figure 3d), activation of hFOXM1 induced expression of genes associated with the JNK and NF-κB pathways, including *Jnk1*, *N-Myc*, *Ccnd1*, *Pttg1*, *Cdkn2a*, *IL1rn*, *Ikbkb*, *Nfkb2* and *Rela* (Figures 4b and c). Chromatin immunoprecipitation assay demonstrated that the FOXM1 protein directly bound to promoter regions of *N-Myc*, *Pttg1*, *Cdkn2a*, *Ikbkb* and *Nfkb2* genes (Figure 4d), indicating a direct transcriptional regulation. FOXM1 did not bind to the *Nfkb1* promoter region (Figure 4d). Thus, FOXM1 overexpression in respiratory epithelial cells is sufficient to induce genes associated with the JNK and NF-κB signaling pathways.

In summary, our data demonstrate that FOXM1 is induced in alveolar type II cells before the formation of lung tumors. Deletion of *Foxm1* prevented the initiation of lung tumors by oncogenic K-Ras^{G12D}. FOXM1 is critical for K-Ras^{G12D}-mediated activation of the NF-κB and JNK signaling pathways in alveolar type II cells. *Ikbkb*, *Nfkb2*, *Pttg1*, *N-Myc* and *Cdkn2a* are novel transcriptional targets of FOXM1.

ABBREVIATIONS

Cre, Cre recombinase; Dox, doxycycline; Fox, Forkhead Box transcription factor; NF-κB, nuclear factor-κB; NSCLC, non-small-cell lung cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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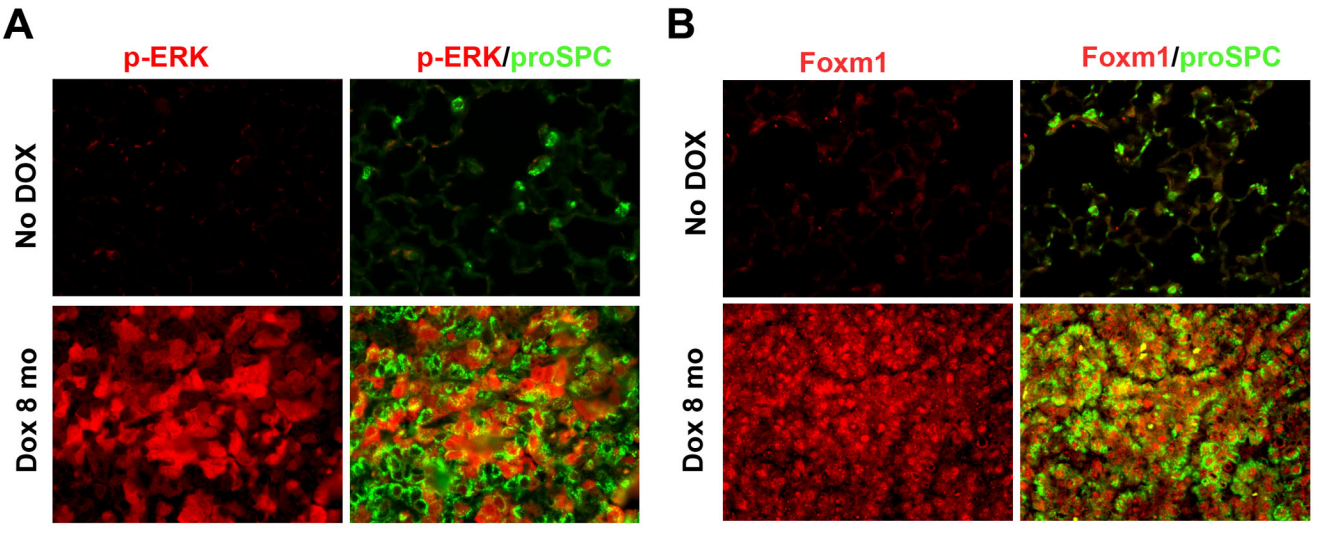
Supplementary Information accompanies this paper on the Oncogene website (http://www.nature.com/onc)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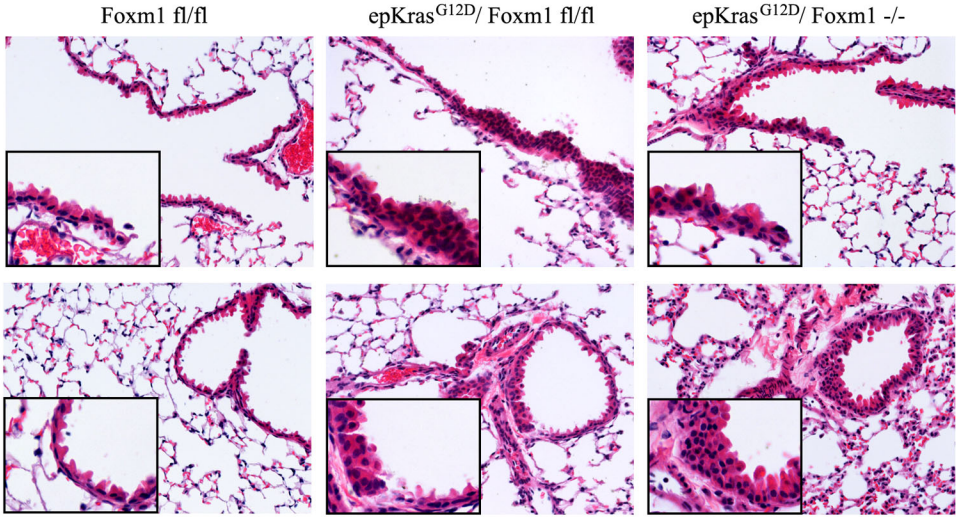
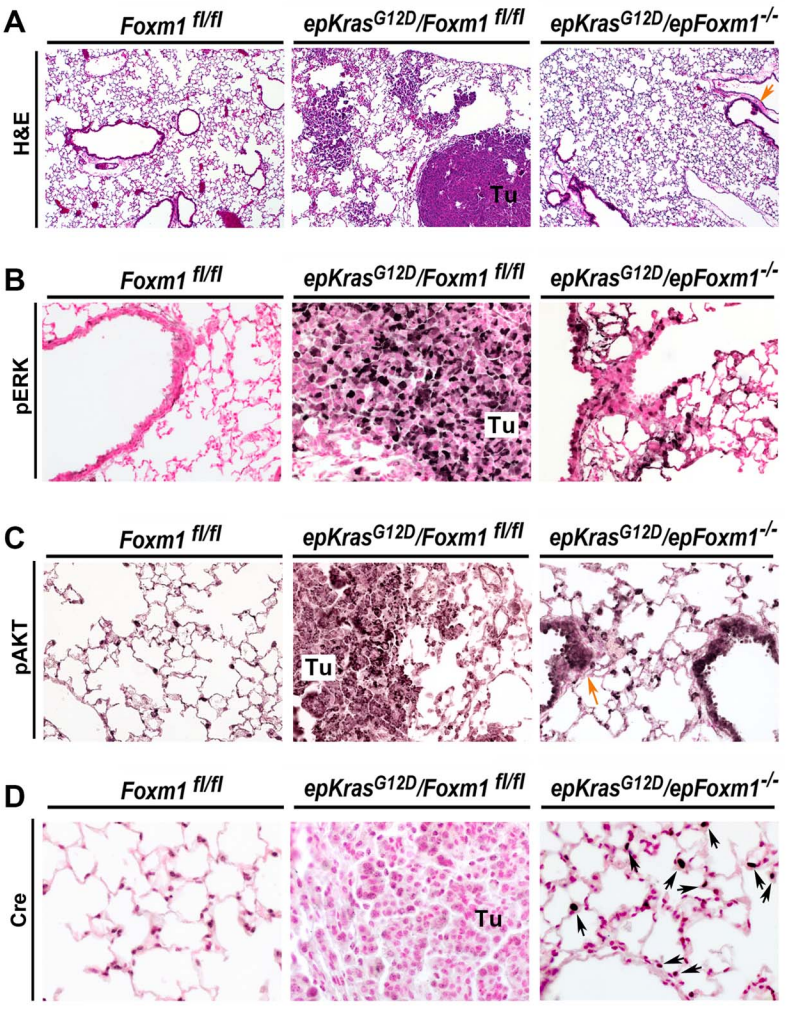
p-ERK
p-ERK/proSPC
No DOX
Dox 8 mo
B
Foxm1
Foxm1/proSPC
No DOX
Dox 8 mo


Foxm1 fl/fl
epKras^{G12D}/ Foxm1 fl/fl
epKras^{G12D}/ Foxm1 -/-


A
Foxm1 fl/fl
epKrasG12D/Foxm1 fl/fl
epKrasG12D/epFoxm1-/-
H&E
Tu
B
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epKrasG12D/epFoxm1-/-
pERK
Tu
C
Foxm1 fl/fl
epKrasG12D/Foxm1 fl/fl
epKrasG12D/epFoxm1-/-
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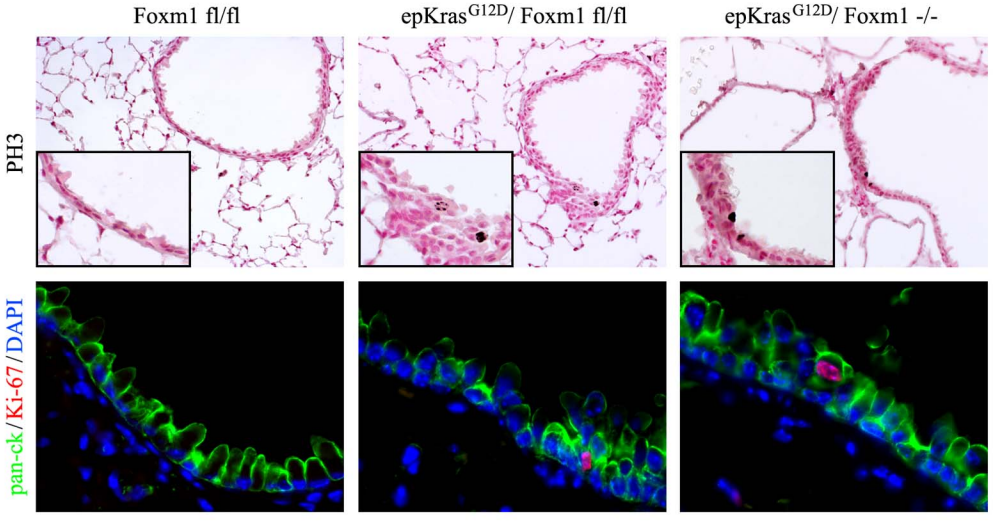
Cre
Tu


Foxm1 fl/fl
epKras^{G12D}/ Foxm1 fl/fl
epKras^{G12D}/ Foxm1 -/-
PH3
pan-ck/Ki-67/DAPI

SUPPLEMENTAL MATERIALS AND METHODS

Transgenic mice. *SPC-rtTA/tetO-Cre/Foxm1*$^{fl/fl}$ mice [1] were bred with *tetO-Kras*^{G12D} mice (Jackson laboratory) to generate *SPC-rtTA/tetO-Kras*^{G12D}*/tetO-Cre/Foxm1*$^{fl/fl}$ quadruple transgenic mice (mixed C57BL/6 and FVB/N genetic background). Generation of *SPC-rtTA/tetO-hFOXM1* mice was described previously [2]. Mice were maintained in pathogen-free vivarium filtered cages. Sentinel mice were free of common viral and bacterial pathogens. Oral Doxycycline (Dox) was given in mouse chow as described [2, 3]. Animal studies were reviewed and approved by Animal Care and Use Committee of the Cincinnati Children's Hospital Medical Center.

Micro-computer tomography (microCT). Mice were euthanized with sodium pentobarbital injection and then lungs were inflated with air prior to imaging. MicroCT data sets were collected using a MicroCAT II scanner (Siemens Medical Solutions USA, Inc. Knoxville, TN). For each scan, 600-605 x-ray projections were collected over a total gantry rotation of 200 degrees. For each projection, the x-ray tube voltage, current, and exposure times were 80 kVp, 500A and 650 - 825 msec, respectively. Each scan required approximately 12 minutes to complete. The CT data sets were reconstructed using a volumetric (cone beam) reconstruction algorithm (COBRA 5.0, Exxim Computing Corp., Pleasanton, CA). Post processing of the CT data sets was done using Amira 5.2 (Visage Imaging, Inc., San Diego, CA). All microCT images had an isotropic resolution of 53 microns. Tumor locations were determined using the orthoslice-viewing module. Tumor volumes were subsequently measured using the segmentation editor. Lung tissue was also segmented to provide 3D surfaces.

Immunohistochemical staining. Paraffin (5 μm) sections were stained with hematoxylin and eosin (H&E) for morphological examination or immunostained with antibodies against Foxm1 (1:2000; clone K-19; Santa Cruze Biotechnology, Santa Cruze, CA), CRE (1:10,000; #69050; Novagen), proSP-C (1;1,500; AB-3428, Chemicon International), pERK (1:2000; Cat#4370; Cell Signaling) or pAKT (1:500; Cat#3787; Cell Signaling). Antibody-antigen complexes were detected using biotinylated secondary antibody followed by avidin-horseradish peroxidase (HRP) complex, and DAB substrate (all from Vector Lab, Burlingame, CA) as described [3]. Sections were counterstained with nuclear fast red

(Vector Labs, Burlingame, CA). Immunofluorescent staining of lung paraffin sections was described previously [2]. Fluorescence was detected using a Zeiss Axioplan 2 Imaging Universal Microscope with an Axiocam MRm digital camera (Axiovision Release 4.3).

Quantitative real-time RT-PCR (qRT-PCR). Total RNA was prepared from lung tissue or purified alveolar type II epithelial cells [4] using RNA Stat-60 (Tel-Test "B" Inc) and analyzed by qRT-PCR using the StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA). Samples were amplified with Taqman Gene Expression Master Mix (Applied Biosystems) combined with inventoried Taqman gene expression assays. Following Taqman gene expression assays were used: mouse genes: Foxm1, Mm00514924_m1; Cdc25b, Mm00499136_m1; Plk1, Mm00440924_g1; c-Myc, Mm00487804_m1; Cxcr2, Mm00438258_m1; Pparg, Mm00440945_m1; Saa1, Mm00656927_g1; Zeb2, Mm00497193_m1; IL1b, Mm01336189_m1; Cdkn1a, Mm01303209_m1; Jnk1, Mm00489514_m1; cJun, Mm00495062_s1; N-Myc, Mm00476449_m1; Atf2, Mm01276558_m1; Pttg1, Mm00479224_m1; Cdkn2a, Mm00494449_m1; Cdh1, Mm00486906_m1; IL1rn, Mm01337566_m1; Ikbkb, Mm01222247_m1; Nfkb2, Mm00479807_m1; Nfkb1, Mm00476361_m1; Rela, Mm00501346_m1; Sqstm1, Mm00448091_m1; Ttf1, Mm00447558_m1; Sftpb, Mm00455681_m1; Gpr65, Mm00433695_m1; Plunc, Mm00465064_m1; Angptl3, Mm00803820_m1; β-actin, Mm00607939_s1. Human FOXM1 mRNA was detected using Taqman gene expression assay Hs00153543_m1. Reactions were analyzed in triplicates and expression levels were normalized to β-actin mRNA.

Chromatin Immunoprecipitation (ChIP) assay. ChIP assay was performed using *in situ* cross-linked human lung epithelial Beas-2B cells as described [5]. Rabbit anti-FOXM1 Ab (C-20, Santa Cruz) and control rabbit IgG (Vector Lab) were used for ChIP [5]. Canonical FOXM1-binding sites in various promoter regions were identified using MacVector program. Primers were designed to avoid repetitive DNA sequences using UCSC In-Silico PCR tool (http://genome.ucsc.edu/cgi-bin/hgPcr?db=hg18). The following sense (S) and antisense (AS) PCR primers were used to amplify promoter DNA fragments in ChIP assay: *N-Myc* (-2236/ -2027) (S) 5'-GCTCCGCTTTCTGCTCAG-3' and (AS) 5'-AGGGTAGTCCGAAGGTGC-3'; *Pttg1* (-2455/ -2329) (S) 5'-CCTGCCTCAATAAAATAGCCCAAC-

3' and (AS) 5'-CACTGTTTCTTCATCTACTGCCCTG-3'; *Cdkn2a* (-2457/ -2163) (S) 5'-CCGACATCGTTTTTCTTCCAG-3' and (AS) 5'-TTCCCTTTTCAGGCACCC-3'; *Ikbkb* (-1392/ - 1293) (S) 5'-CCAAGCAGGAAGGAGATGAAC-3' and (AS) 5'- CCTAGCAGTGTGAAGCCAAAC-3'; *Nfkb2* (-3956/ -3774) (S) 5'-CTGAGATAGGGAAAAACCGC-3' and (AS) 5'-CACAAGACAATAGTGGGGAGAG-3'; *Spdef* (-120/ +58) (S) 5'-CCTGCAAGGGTTAATCAGGAGCCT-3' and (AS) 5'- GCAGTGTGGACACGGCAGAGTGCA-3'; *Nfkb1* (-3744/ -3528) (S) 5'-GGCATTGTCACACAGGTTC-3' and (AS) 5'-GCATCTCTTTTCCCAGTAGTG-3'.

Statistical analysis. Post-hoc ANOVA was used for multiple group comparison. For comparison between two groups, Student's T-test was used to determine statistical significance. P values ≤ 0.05 were considered significant. Values for all measurements were expressed as the mean ± standard deviation (SD).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Foxm1 and pERK are co-expressed with proSP-C in SPC-rtTA/ TetO-Kras^{G12D} tumors. Lungs from SPC-rtTA/ TetO-Kras^{G12D} mice, that were given Dox for 8 months, were paraffin-embedded, sectioned and stained with antibodies against Foxm1, pERK and proSP-C. proSP-C co-localizes with p-ERK (A) and Foxm1 (B) in lung tumors of Dox-treated SPC-rtTA/ TetO-Kras^{G12D} mice. Neither pERK nor Foxm1 were found in lungs of SPC-rtTA/ TetO-Kras^{G12D} mice without Dox. Magnifications are x400.

Supplemental Figure 2. Deletion of *Foxm1* from *Kras^{G12D}*-expressing respiratory epithelium does not influence epithelial hyperplasia. Lung paraffin sections from Dox-treated epKras^{G12D}/ ep*Foxm1*$^{-/-}$, epKras^{G12D}/ *Foxm1*$^{fl/fl}$ and *Foxm1*$^{fl/fl}$ mice were stained with H&E. Dox was given for 4 months.

Epithelial hyperplasia is observed in bronchiolar epithelium of epKras^{G12D}/ *Foxm1*$^{fl/fl}$ and epKras^{G12D}/ ep*Foxm1*$^{-/-}$ mice, but not in *Foxm1*$^{fl/fl}$ mice. Magnifications are x200 and x400 (inserts).

Supplemental Figure 3. pERK and pAKT are induced in hyperplastic lung epithelium of epKras^{G12D}/ ep*Foxm1*$^{-/-}$ mice. Paraffin sections of lungs from Dox-treated epKras^{G12D}/ ep*Foxm1*$^{-/-}$, epKras^{G12D}/ *Foxm1*$^{fl/fl}$ and *Foxm1*$^{fl/fl}$ mice were stained with H&E (A) or used for immunohistochemistry with antibodies against phospho-ERK (Thr202/Tyr204) (B), phospho-AKT (Ser437) (C) and Cre (D). Dox was given for 8 months (n=3 mice in each group). Slides were counterstained with Nuclear Fast Red (red nuclei). pERK and pAKT were increased in lung tumors (Tu) and hyperplastic lung epithelium (yellow arrows). Positive staining for Cre is shown with black arrows in D. Magnification: x50, A; and x400, B-D.

Supplemental Figure 4. Increased cellular proliferation in hyperplastic epKras^{G12D}/ ep*Foxm1*$^{-/-}$ bronchiolar epithelium. Lungs from Dox-treated epKras^{G12D}/ ep*Foxm1*$^{-/-}$, epKras^{G12D}/ *Foxm1*$^{fl/fl}$ and *Foxm1*$^{fl/fl}$ mice, that were given Dox for 4 months, were paraffin-embedded, sectioned and stained with antibodies against PH3 (top panels). Nuclear fast red was used for counterstaining. PH3-positive cells are detected in hyperplastic epithelial regions of epKras^{G12D}/ *Foxm1*$^{fl/fl}$ and epKras^{G12D}/ ep*Foxm1*$^{-/-}$ bronchioles. Ki-67 (red) is expressed in hyperplastic epithelial cells that are positive for epithelial cytokeratin (CK, green) (bottom panels). Magnifications are x200 (top panels), x400 (inserts) and x800 (bottom panels).

SUPPLEMENTAL REFERENCES

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