Activated Notch1 Induces Lung Adenomas in Mice and Cooperates with Myc in the Generation of Lung Adenocarcinoma

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Abstract

Notch1 encodes the canonical member of the mammalian Notch receptor family. Activating lesions frequently affect Notch1 in T-cell acute lymphoblastic leukemia (T-ALL) and, recently, have been found in non–small-cell lung cancer (NSCLC) as well. We explored the oncogenic potential of activated Notch1 in the lung by developing a transgenic mouse model in which activated Notch1 was overexpressed in the alveolar epithelium. The initial response to activated Notch1 was proliferation and the accumulation of alveolar hyperplasia, which was then promptly cleared by apoptosis. After an extended latency period, however, pulmonary adenomas appeared in the transgenic mice but failed to progress to become carcinomas. Interestingly, Myc and MycL1 were expressed in the adenomas, suggesting that selection for enhanced Myc activity may facilitate tumorigenesis. Using mice engineered to coexpress activated Notch1 and Myc, we found that supplementing Myc expression resulted in increased frequency of Notch1 intracellular domain (N1ICD)-induced adenoma formation and enabled progression to adenocarcinoma and metastases. Cooperation stemmed from synergistic activation of tumor cell cycling, a process that apparently countered any impedance to tumorigenesis posed by Myc and/or activated Notch1-induced apoptosis. Significantly, cooperation was independent of RAS activation. Taken together, the data suggest that activated Notch1 substitutes for RAS activation synergistically with Myc in the development of NSCLC. These tumor models should be valuable for exploring the role of activated Notch1 in the genesis of NSCLC and for testing therapies targeting either activated Notch1 or its downstream effectors. Cancer Res; 71(18); 1–9. ©2011 AACR.

Introduction

The Notch signaling pathway functions in cell-fate determination and differentiation (1). There are 4 Notch genes in the mammalian genome (Notch1–4 in humans). The genes encode single-pass transmembrane proteins that interact with ligands of the Delta and/or Jagged/Serrate family. The ligands are also transmembrane proteins, which bind to Notch receptors displayed on adjacent cells. Ligand binding induces proteolytic cleavage of the Notch receptor and release of the Notch intracellular domain (NICD; refs. 2, 3). This is the activated form of Notch, which enters the nucleus and functions as a transcriptional coactivator for DNA-binding transcription factors of the CBF1/SU(H)/LAG1 (CSL) family (4). Notch target genes are normally repressed by the CSL family of proteins, but become activated through the binding of a NICD/CSL complex and its recruitment of chromatin remodeling proteins (5).

A role for Notch signaling in cancer was first suspected with the characterization of t(7;9)(q34;q34.3) chromosomal translocations in a subset of human T-cell acute lymphoblastic leukemia (T-ALL; ref. 6). The translocation breakpoints occur in an intron of the Notch1 gene and result in the formation of an mRNA encoding a truncated and constitutively active Notch1 intracellular domain (N1ICD) protein fragment. It is now known that point mutations and small, frame-shifting insertions or deletions in Notch1 also occur in T-ALL (7). These alterations enable either ligand-independent cleavage combined with N1ICD release or interrupt the domain that regulates N1ICD turnover, the C-terminal PEST domain, resulting in increased Notch1 signal. It has been suggested that Notch alterations are uncommon in malignancies other than T-ALL (6), but activating alterations in Notch1 have been described in approximately 10% of non–small-cell lung cancer (NSCLC; ref. 9). An additional 30% of NSCLCs have lost expression of Numb, a negative regulator of Notch, resulting in increased Notch activity (9).
To explore the tumorigenic potential of activated Notch1, we targeted the expression of an N1ICD transgene to the pulmonary epithelium. Expression of the transgene could be induced by administration of doxycycline. Continuous N1ICD overexpression in the alveolar epithelium induced lung adenomas, but not adenocarcinomas. Progression of lung adenomas overexpressing N1ICD to adenocarcinoma could be brought about by the additional overexpression of Myc, which cooperated with N1ICD through synergetic effects on tumor cell proliferation. Previously, activating mutations in Kras were found in tumors from a murine model of NSCLC that overexpresses Myc (10). The cooperation we observed between N1ICD and Myc was independent of Ras activation, suggesting that activated Notch1 can substitute for activated Ras in tumorigenesis with Myc. Our results authenticate the tumorigenic potential of activated Notch1 in the lung, particularly in combination with Myc, and raise the possibility that therapeutics targeting Notch1 may prove valuable in the treatment of human NSCLCs that express activated forms of the protein.

Materials and Methods

Genetically modified mice
Experimentation was conducted with the approval of the Institutional Animal Care and Use Committee of the University of California, San Francisco, CA. Mice expressing the doxycycline-responsive reverse tetracycline transactivator (rtTA) under the control of the rat Clara cell secretory protein (CCSP) promoter (11) and mice that have a tetracycline-responsive promoter element that controls the transcription of human N1ICD (12) have both been previously described. Transgenic mice with a tetracycline-responsive promoter element that controls the transcription of the human MYC sequence were developed in our laboratory (13). A diet supplemented with doxycycline (200 mg/kg) was used to stimulate the transactivating function of the rtTA protein. Lung phenotypes were evaluated in accordance with the criteria recommended by the Mouse Models of Human Cancer Consortium (14). Under these recommendations, adenocarcinomas are defined as tumors ≥5 mm in diameter, while adenoma, considered the precursor to adenocarcinoma, is <5 mm in diameter.

Western blot analysis
Snap-frozen lung and tumor tissues were used to prepare protein lysates, and Western blot analysis was carried out using standard techniques. Sources of antibodies used for detection are listed in Supplementary Materials and Methods.

Tissue staining
Immunohistochemical staining was carried out using the Vector Elite ABC Kit (Vector Laboratories). Primary antibodies used for staining are listed in Supplementary Materials and Methods. We used the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon) for terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL staining). For the quantification of cells positive for phosphorylation of histone-3 at serine-10 (phospho-H3S10), Ki67, or TUNEL staining, at least 3 random, hps were counted for each individual lung or tumor (n ≥ 3 mice for each genotype).

TaqMan analysis
RNA was extracted using the Absolutely RNA Miniprep Kit (Stratagene). RNA was reverse-transcribed using StrataScript reverse transcriptase (Stratagene) and analyzed by real-time PCR (TaqMan; Applied Biosystems). Relative gene expression was normalized to a mouse β-actin TaqMan probe using the ΔCt method (n ≥ 3 mice for each analysis).

RAS activity assay
RAS activity was measured in 250 μg of protein lysate using the RAS Activation Assay Kit (Millipore). In brief, the kit supplied a GST-RAF RAS-binding domain fusion protein to precipitate only ATP-bound RAS from protein lysate.

Results

Conditional expression of activated Notch1 in the alveolar epithelium
To model Notch1 activation in the lungs of adult mice, we overexpressed N1ICD using a doxycycline-inducible system (Fig. 1A). Transgenic mice expressing rtTA under the transcriptional control of the rat CCSP promoter (the C transgene) were used to achieve lung-specific expression. Although CCSP is expressed in Clara cells of the bronchiolar epithelium, the C transgene is transcriptionally active mainly in epithelial cells of the distal lung (11). We bred C mice to mice carrying a transgene encoding N1ICD (sequence of human Notch1 encoding amino acids 1,756–2,556) under the transcriptional control of a tetracycline-response element (TRE) promoter (the N1 transgene; ref. 12). A diet supplemented with doxycycline was used to stimulate the transactivating function of the rtTA protein and induce N1ICD expression in the resulting CN1 mice. The 110-kDa N1ICD protein fragment was induced in CN1 preparations (Fig. 1B). N1ICD levels decreased after day 14 but, nonetheless, remained elevated (see day 30; Fig. 1B). We assayed the mRNA levels of a subset of known targets of the Notch transcriptional complex. Some responded to doxycycline induction, including Hes5 and Nrarp (Fig. 1C). Notably, Hes1, a gene whose transcription is often used as a measure of Notch activation, was not induced. Therefore, N1ICD overexpression activated only a subset of the known Notch targets, presumably reflecting tissue-specific differences in the transcriptional program activated by Notch.

N1ICD induction of alveolar hyperplasia and apoptosis
We intended to assess whether there were any acute consequences associated with N1ICD overexpression. Histologic
examination of hematoxylin and eosin (H&E)-stained sections revealed an abundance of alveolar hyperplasia after 7 days of doxycycline treatment (compare Fig. 1D with 1E). The hyperplasia, composed of clusters of cells found in the alveolar space, reached a maximum after 14 days of doxycycline treatment (Fig. 1F) and regressed thereafter (Fig. 1G). Regression was never absolute as hyperplastic cells persisted in the lungs of older mice (compare Fig. 1D with 1G). Immunohistochemical staining for Notch1 confirmed that the hyperplastic cells overexpressed N1ICD (Fig. 1H–K).

We hypothesized that the transient hyperplasia could be attributed to proliferation followed by apoptosis. Immunohistochemical staining for Ki67 antigen was done to mark cycling cells (Supplementary Fig. S1A–D). The number of cycling cells in doxycycline-treated CN1 mice was substantially increased after 7 and 14 days of treatment, but regressed by 30 days of continuous doxycycline treatment (Fig. 2A). TUNEL staining was used to detect apoptotic cells with fragmented DNA (Supplementary Fig. S1E–H). CN1 mice did not have a substantial increase in TUNEL + cells after 7 days of continuous doxycycline treatment, but positive cells were abundant after 14 days and remained elevated after 30 days (Fig. 2B). Therefore, alveolar epithelial cells proliferated in response to N1ICD overexpression, but cell death tempered the proliferative response.

**N1ICD overexpression engages the Bcl-2 family of apoptotic regulators**

To define mediators of the apoptosis that cleared N1ICD-induced alveolar hyperplasia, we screened protein lysates from doxycycline-treated CN1 mice for alterations in anti- and pro-apoptotic proteins. We found that the anti-apoptotic Bcl-2 protein was repressed in CN1 mouse lungs after 14 days of doxycycline treatment (Fig. 2C). Induction of full-length, pro-apoptotic Bcl-2 family proteins Bak and Bok and the BH3-only proteins Puma, Bik, Bmf, and Bim, accompanied Bcl-2 repression (see Supplementary Fig. S2A for unaltered Bcl-2 family proteins). Concurrently, cleaved forms of caspases 3 and 7 and the caspase target PARP1 accumulated. We concluded that activated Notch1 induced alterations in multiple Bcl-2 family proteins to stimulate the intrinsic apoptotic cascade.

Both *Puma* (15) and *Bok* (16) are transcriptional targets of the p53 tumor suppressor. We reasoned that p53 induction might contribute to the apoptotic clearance of N1ICD-induced alveolar hyperplasia, but expression analysis of mRNAs encoding Bcl-2 family proteins altered by N1ICD showed a modest but statistically significant induction of *Bik* and *Bim*, but not *Puma* or *Bok* (Supplementary Fig. S2B). In addition, neither p53 protein nor *trp53* mRNA was induced at day 14 of doxycycline treatment (Supplementary Fig. S3A and B). We concluded that apoptotic signaling was engaged independent...
of p53 induction and that nontranscriptional regulatory mechanisms, which are yet to be defined, likely account for most of the observed alterations in Bel2 family proteins.

**NIICD-induced lung adenomas**

We reasoned that, over time, genetic and/or epigenetic defects might accumulate in the lungs of CN1 mice, enabling cells to evade intrinsic tumor-suppressive functions, such as apoptosis. Therefore, we monitored doxycycline-fed CN1 mice at different time-points for signs of tumorigenesis. Beginning as early as at 8 months of age, we observed that N1ICD-overexpressing cells expanded laterally along the alveolar walls in a bronchioalveolar pattern (Fig. 3A and B). Small adenomas could be found in each of the 8-month-old CN1 mice (n = 4 mice). These early tumors had bronchioalveolar patterning (Fig. 3C) and appeared to have been formed from the coalescence of N1ICD+ cells (Fig. 3D).

Eventually, doxycycline-fed CN1 mice developed multiple adenomas with papillary histology (Fig. 3E) and succumbed to their tumor burden at an average age of 15.1 ± 2.2 months (n = 11 mice). We did not find adenocarcinomas (size ≥ 5 mm) in any of these mice. Only rare adenomas were found in CN1 mice that were not fed a doxycycline diet (average = 0.38 ± 0.72 per mouse; n = 16 mice).

We investigated the expression of markers of differentiated lung epithelial lineages in alveolar hyperplasia and the adenomas that arose in doxycycline-fed CN1 mice. Alveolar hyperplasia in CN1 mice treated with doxycycline for 7 days was thyroid transcription factor-1 (TTF-1) and surfactant protein C (SPC) positive (Supplementary Fig. S4A–C), suggesting that it may have arisen from type II pneumocytes, where TTF-1 and SPC are coexpressed. Adenomas from CN1 mice were TTF-1+/− and CCSP− with scattered SPC+ cells (Fig. 3F–H). Therefore, both the expression of SPC and the anatomic location in which N1ICD+ cells first arise suggest that N1ICD+ adenomas may derive from type II cells.

**The Arf-Mdm2–p53 network in NIICD-induced adenomas**

We speculated as to what other factors contributed to the formation of lung adenomas in doxycycline-treated CN1 mice. For example, in T-ALL, NIICD suppresses the Arf–Mdm2–p53 network (12). However, examination of the Arf–Mdm2–p53 network in the lungs of CN1 mice showed that p19Arf was induced, not repressed, at both the protein and mRNA level when NIICD was overexpressed (Supplementary Figs. S3A and S5A). In addition, Mdm2 was transcriptionally repressed in adenomas (Supplementary Fig. S2B). Both findings are consistent with stabilization, not degradation, of p53, although no p53 accumulation was observed (Supplementary Fig. S3A). Our data suggests that, in the lung, NIICD does not inhibit p53 through repression of p19Arf, a finding that contrasts with findings in T-ALL (12).

**Myc and MycL1 are induced in adenomas**

Furthermore, we examined the hypothesis that long-term formation of adenomas in CN1 mice could be mediated, at least in part, by Myc activity. Transcription from Myc is induced by activated Notch1 in T-ALL (17, 18) and in mammary tumors (19). We measured expression of Myc, MycL1, and MycN in lung protein extracts from CN1 mice treated with doxycycline. Myc and, to a lesser extent, MycL1 were induced in the lungs of mice treated with doxycycline for 7 and 14 days (Fig. 4A). The expression of both proteins was undetectable by day 30 of doxycycline treatment by which time hyperplasia had mostly resolved. However, expression of Myc and MycL1 reemerged in adenomas from CN1 mice receiving long-term treatment with doxycycline (Fig. 4A).

Next, we measured the mRNA level of the Myc genes in the lungs and adenomas of CN1 mice. Although Myc and MycL1 protein could be easily detected after just 7 days of doxycycline treatment, no changes in the transcription of any of the Myc genes was detected (Fig. 4B). Adenomas, however, had upregulated transcription of both Myc and MycL1, but not MycN, a result that mirrored changes observed at the protein level.
Therefore, transcriptional induction of both Myc and Mycl1 could account for the presence of Myc and MycL1 in adenomas, but altered posttranscriptional regulation likely underlies the upregulation of Myc and MycL1 protein in N1ICD-induced alveolar hyperplasia. Nonetheless, we concluded that Myc and MycL1 expression reemerged in adenomas, presumably in response to selection that favored tumorigenesis.

**Myc and N1ICD cooperate in lung tumorigenesis**

To test whether sustained Myc activity could facilitate the formation of N1ICD-induced adenomas, we crossed CN1 mice to mice carrying a doxycycline-regulatable Myc transgene (13; the M transgene) and created compound CN1M mice (schematic in Fig. 5A). We found that mice overexpressing both N1ICD and Myc had dramatically decreased survival rate (Fig. 5B) when compared with mice overexpressing either N1ICD (CN1 mice) or Myc (CM mice) alone.

Tumorigenesis was always multifocal in doxycycline-fed CN1 mice (35.6 ± 17.5 tumors visible on the pleural surface, n = 11; Fig. 5C and D), but the tumors in CN1 mice were always adenomas, never adenocarcinoma. In doxycycline-fed CN1M mice, we also observed multifocal tumorigenesis...
However, not only were there more tumors in CN1M mice (113.3 ± 45.4 tumors visible on the pleural surface; n = 10) but large adenocarcinomas, as well as adenomas, were found in each mouse. A significant proportion of CN1M mice harbored gross metastases (4/13, 30.8%; Fig. 5F–H). Metastatic cells were observed in enlarged lymph nodes, the liver, and lining the walls of the thoracic cavity. TTF-1 staining confirmed the lung origin of distant metastases (Fig. 5G and H).

We previously reported that a high percentage of CM mice succumb to lung adenocarcinomas (10). Usually, a single adenocarcinoma grew to occlude the parenchymal space (Fig. 5I) before symptoms of respiratory distress were observed (1.3 ± 1.1 tumors per mouse; n = 31). The data suggest that the combination of activated Notch1 and Myc induced a more aggressive lung tumor phenotype when compared with mice expressing activated Notch1 or Myc alone.

### N1ICD substitutes for activated RAS in lung tumors overexpressing Myc

Western blot analysis of tumor protein lysates suggested that even more of the 110-kDa N1ICD was present in CN1M adenocarcinomas than in CN1 adenomas (Fig. 6A). Myc overexpression may, therefore, stabilize N1ICD, although a mechanism by which this might occur is not apparent. We concluded that both N1ICD and Myc were overexpressed in CN1M adenocarcinomas.

Like the levels of N1ICD, Hes5 induction was highest in CN1M adenocarcinomas (Fig. 6B), although the difference in expression compared with CN1 adenomas was not statistically significant. Other Hes and Hey genes were transcriptionally repressed in CN1M adenocarcinomas compared with CN1 adenomas (Supplementary Fig. S6). This reinforces the supposition that Hes5 is a target of N1ICD in the lung epithelium, whereas other Hes and Hey basic helix–loop–helix genes may not be.

Previously, we have shown that lung tumors from CM mice harbor mutations in Kras that cooperate with Myc in tumorigenesis (10). Cross-talk between Notch1 and RAS has been suggested to be important for transformation (20, 21) and, in some mouse tumor models, activated Notch1 cooperates with activated Kras (22, 23). We hypothesized that cooperativity between N1ICD and Myc in lung tumorigenesis may require RAS activation and carried out a RAS activity assay on protein...
lysates of tumors from CN1, CN1M, and CM mice (Fig. 6C). No increase in RAS activity was seen in CN1 adenomas or CN1M adenocarcinomas. This suggests not only that RAS activation is not required for the formation of N1ICD-induced adenomas, but also that N1ICD can substitute for mutation of Kras in a cooperative process with Myc that produces lung adenocarcinoma.

N1ICD and Myc have a synergistic effect on tumor cell proliferation

Acute overexpression of Myc induces apoptosis in a variety of tissues (24), including the lung (10). Compensatory genetic and/or epigenetic events are thought to counter the pro-apoptotic effects of Myc during tumorigenesis (25, 26). Given that overexpression of either N1ICD or Myc can elicit an antitumor response in the form of apoptosis, we wondered how cooperativity was achieved during lung tumorigenesis.

Tumors from CN1, CN1M, and CM mice were immunostained for phospho-H3S10, a marker of cells in the mitotic phase of the cell cycle (Supplementary Fig. S7A–C). We observed a significant increase in mitotic cells in CN1M adenocarcinomas as compared with both CN1 adenomas (3.7-fold) and CM adenocarcinomas (3.3-fold; ref. Fig. 6D). We observed similar changes with immunostaining for Ki67 antigen (data not shown), which stains cells in all phases of the cell cycle. Therefore, the combination of N1ICD and Myc expression had a synergistic effect on tumor cell cycling.

In addition, we observed an increase in TUNEL staining in adenocarcinomas from CN1M mice compared with CN1 adenomas (3.2-fold) and CM adenocarcinomas (2.8-fold; Fig. 6E; Supplementary Fig. S7D–F). Therefore, the combination of N1ICD and Myc expression also augmented the induction of apoptosis. However, because the fold increase in phospho-H3S10+ cells was higher than the increase in
TUNEL$^+$ cells, the pro-proliferative effect of coexpressing activated Notch1 and Myc outcompeted apoptosis induction. Thus, synergistic activation of tumor cell cycling contributed to the cooperative effect of N1ICD and Myc on lung tumorigenesis.

Discussion

N1ICD induction of apoptosis in the alveolar epithelium

We used a doxycycline-inducible system to overexpress N1ICD in the lungs of adult mice. This resulted in the formation of lung adenomas. However, the initiation of adenomas required prolonged stimulation of the $N1$ transgene. Adenomas were first observed in 8-month-old CN1 mice that had been continuously fed a doxycycline-containing diet. This chronology suggests that additional genetic/epigenetic events that cooperate with N1ICD overexpression must accumulate to enable N1ICD-induced tumorigenesis. The requirement for anti-apoptosis likely contributes to the observed latency.

The effect of activated Notch1 on apoptosis is context dependent. Activated Notch1 protects from apoptosis in some settings (27–29) but acts to induce apoptosis in others (30, 31). We found that in the mouse alveolar epithelium, acute activation of N1ICD overexpression resulted in a wave of proliferation and the formation of extensive hyperplasia, but the bulk of the alveolar hyperplasia was cleared from the lung periphery. The upregulation of multiple BHE3-only proteins, Bak and Bok, and repression of Bcl-2 all occurred, resulting in the activation of the intrinsic apoptotic cascade.

In this setting, the induction of apoptosis may be tumor suppressive, effectively countering the mitogenic response to activated Notch1. Similar observations with regard to the induction and apoptotic clearance of alveolar hyperplasia have been made in transgenic mice with lung-specific expression of Myc (10, 32) that also develop lung tumors following a prolonged latency period. This suggests that the acquisition of genetic/epigenetic events that provide anti-apoptosis may precede lung adenoma formation elicited by activated Notch1 and/or Myc.

Activated Notch1 and Myc cooperate in lung tumorigenesis

On the basis of the reemergence of Myc and MycL1 expression in adenomas, we hypothesized that Myc activity could be important for the initiation of N1ICD-induced lung adenomas. We speculated that more adenomas would form if Myc activity were further supplemented through transgenic manipulation, and we developed mice that could overexpress both N1ICD and Myc coincidentally to test this supposition. These mice did have a significant increase in the number of lung tumors that were initiated, but the mice developed adenocarcinomas as well as adenomas. Some mice even harbored gross metastases. This suggests a robust cooperation between N1ICD and Myc in lung tumorigenesis.

Previously, we showed that activating mutations in $Kras$ are present in Myc transgenic tumors, including CM lung adenocarcinomas (10). In this study, however, we observed that RAS is not activated in CN1M lung tumors. This suggests that activated Notch1 can substitute for activated $Kras$ in tumorigenesis with Myc. It would be worthwhile to compare the frequency and overlap of $Kras$ mutation and activated Notch1 expression in human NSCLCs expressing Myc, as our data suggests $Kras$ mutation and activated Notch1 expression could be mutually exclusive.

Activated Notch1 and Myc synergistically activate tumor cell cycling

In spite of the lack of progression of adenomas to adenocarcinoma in doxycycline-fed CN1 mice, the number of phospho-H3S10$^+$ cells found in adenomas rivaled that found in adenocarcinomas from CM mice, which can grow to occlude entire lobes of the lung and then metastasize (10). It would appear, therefore, that CN1 adenomas harbor a significant number of cells that can actively enter the cell cycle. Apoptotic cells, however, were also prevalent in CN1 adenomas. We infer that apoptosis may limit the progression of CN1 adenomas to adenocarcinoma, although other means of tumor suppression might also contribute to this.

The inference that apoptosis limited progression of adenomas to adenocarcinoma might appear paradoxical, as apoptosis was still prevalent in adenocarcinomas from doxycycline-fed CN1M mice. In fact, N1ICD and Myc coexpression induced a synergistic increase in apoptosis, as one might expect with 2 oncoproteins that can induce an apoptotic response. However, N1ICD and Myc coexpression also synergized in the induction of cell cycling. The fold-increase in mitotic cells was higher than the fold-increase in apoptotic cells. The observed synergy is in direct contrast to recent findings in a murine model of T-ALL, where supplementing the expression of Myc lent no additional advantage to malignant cells that overexpress N1ICD (33). Our findings imply that small differences in the ratio of proliferating to apoptotic cells can have profound effects on lung tumor progression and survival, an implication consistent with studies investigating the prognostic significance of apoptotic and proliferative indices in human NSCLC (34).

To our knowledge these are the first transgenic models to be reported wherein lung tumors are directly induced by activated Notch1. The CN1 and CN1M models will be valuable for dissecting the genes and pathways that mediate the contribution of activated Notch1 to tumorigenesis in the lung. In addition, they may be valuable for the preclinical evaluation of therapeutics targeting either activated Notch1 or targets of Notch1 that are important for transformation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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