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Secretoglobin 3A2/uteroglobin-related protein 1 is a novel marker for pulmonary carcinoma in mice and humans

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Abstract

Secretoglobin (SCGB) 3A2, also called uteroglobin-related protein (UGRP) 1, is a downstream target for a homeodomain transcription factor NKX2-1, which is critical for the development of lung, thyroid and ventral forebrain. Both SCGB3A2 and NKX2-1 are expressed in airway epithelial cells and the latter also in alveolar Type II cells. NKX2-1 has been used clinically for diagnosis of human pulmonary tumors. Recently, the expression of SCGB3A2 was reported in human carcinomas, suggesting the use of this protein as a tumor marker. In this study, twenty eight lung tumors from aging B6;129 mice and nine lung adenocarcinomas from CC10TAg transgenic mice that express SV40 large T antigen under the mouse *Scgb1a1* (*CC10*) gene promoter, were subjected to histopathological and immunohistochemical analyses for the expression of NKX2-1 and SCGB3A2. NKX2-1 was expressed in all types of tumors albeit more focally in carcinomas. In contrast, SCGB3A2 normally expressed in Clara cells, was negative in Type II cell hyperplasias and adenomas. However, it was expressed in alveolar Type II cell carcinomas and Clara cell adenocarcinomas. In these carcinomas, SCGB3A2 expression was observed in the portion of the tumor where NKX2-1 expression was reduced or almost abolished. As a comparison, the expression of SCGB3A2 and NKX2-1 from twenty-three human non-small cell lung carcinoma specimens was also examined. The results demonstrate that SCGB3A2 is a useful marker for diagnosis of pulmonary tumors both in mice and humans.

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Keywords

SCGB3A2; UGRP1; NKX2-1; TTF1; NSCLC; aging B6; 129 mice; CC10TAg transgenic mice; pulmonary carcinoma; carcinoma marker; histopathological and immunohistochemical analysis

1. Introduction

Secretoglobin (SCGB) 3A2, previously called uteroglobin-related protein (UGRP) 1, was originally identified as a downstream target for NKX2-1 through suppressive subtractive library screening of mRNAs isolated from lungs of *Nkx2-1*-null vs wild-type mouse fetuses [1]. SCGB3A2 is a member of the SCGB gene superfamily, which is comprised of secretory proteins of small molecular weight of approximately 10 kDa [2]. The most studied member of this gene superfamily is SCGB1A1, also called Uteroglobin, Clara cell specific 10-kDa protein (CC10), or Clara cell secretory protein (CCSP) [3]. Like SCGB1A1 (CC10), SCGB3A2 expression is mainly found in bronchial epithelial cells. SCGB3A2 is first detected in mouse fetal lungs of embryonic day (E) 11.5. Its expression markedly increases by E16.5 and continues in the airway epithelial cells (Clara cells) at relatively high levels throughout adulthood [1,4]. Two major roles for SCGB3A2 have been described as a growth factor during fetal lung development [4] and an anti-inflammatory agent in lung [5]. However, unlike SCGB1A1 (CC10), very little information is available on SCGB3A2 during lung carcinogenesis.

NKX2-1, also called TTF1, TITF1, or T/EBP, is a major transcription factor for the development and differentiation of thyroid, lung, and ventral forebrain [6]. In lung, it regulates expression of genes in airway epithelial cells such as surfactant protein (SP)-A [7], SP-B [8], SP-C [9] and SCGB1A1 (CC10) [10]. These genes serve as important epithelial markers during lung development and differentiation [11-14]. NKX2-1 (TTF1) is a lineage-specific oncogene amplified in lung cancer [15-17], and is expressed in human lung adenocarcinomas and small cell carcinomas at high frequency (62.5 and 92.7%, respectively in [18]; 96 and 89%, respectively in [19]). Clinically, NKX2-1 has been used as a pulmonary tumor marker [18,19]. The surfactant proteins (SP-A, B, C) also serve as tumor markers, however, the sensitivity is lower as compared with NKX2-1 [20-22]. The expression of SP-A, SP-B (or pro-SP-B) and SP-C (or pro-SPC) protein is found in 20-30% of human pulmonary adenocarcinomas as determined by immunohistochemistry, while SP-A and SP-C mRNAs are expressed at 33.3 and 14.1%, respectively in peripheral blood of patients with non-small cell lung carcinomas (NSCLC) as determined by RT-PCR [23]. On the other hand, SCGB1A1 (CC10) is considered to have tumor suppressor properties and is expressed in less than 10 % of human NSCLCs [24]. In mice, expression of SCGB1A1 (CC10) is absent in spontaneous lung tumors and minimal in tumors developed in CC10TAg mouse that expresses SV40 large T antigen under the promoter of mouse *Scgb1a1* (*CC10*) gene [25-28].

Recently, the expression of SCGB3A2 was reported in human lung carcinomas, providing SCGB3A2 as a potential useful tool for diagnosis of pulmonary tumors [29]. The current study was initiated to determine whether SCGB3A2 can be used as a marker for classifying mouse pulmonary tumors. The expression of SCGB3A2 was compared with NKX2-1 expression in neoplastic and non-neoplastic lung lesions. Their expression in human tumors was also examined.

2. Material and Methods

2.1. Tissues

Sources for mouse lung neoplastic tissues were twenty eight aging B6;129 or 129S4/SvJae mice [30,31] and nine 10-16-week old CC10TAg transgenic mice that express SV40 large T antigen under the control of mouse *Scgb1a1* (*CC10*) gene promoter [26]. Aging B6;129 mice (up to 2 years of age) spontaneously developed lung tumors that originated from alveolar Type II cells [30,31] while tumors in CC10TAg mice initially developed after one month of age and were of Clara cell origin (bronchiolar) and adenocarcinomas by histology [25-28]. All animal studies were performed after approval by the National Cancer Institute (NCI) Animal Care and Use Committee. For comparison we included twenty-three routine archival human NSCLC resection specimens, which were obtained through the Internal Review Board-approved protocols. In addition, normal lung specimens from three healthy adults were obtained from the NCI sponsored Cooperative Human Tissue Network (CHTN), Philadelphia, PA.

2.2. Histology

Lungs were fixed in 10% neutral buffered formalin, embedded in paraffin and 4-μm sections were mounted on glass slides. Sections were deparaffinized with xylene and graded ethanol, and stained with hematoxylin and eosin (H&E) for classification of tumors. Immunohistochemical staining for mouse NKX2-1, and mouse and human SCGB3A2 was performed using rabbit antibodies that were produced in our laboratory [1,29,32]. For immunostaining of human tissues for NKX2-1 and SCGB1A1 (CC10), a mouse monoclonal antibody (Clone SPT24) from Leica Microsystems (Bannockburn, IL) and rabbit-anti UP1 (CC10 antibody) from Dako (Carpinteria, CA), respectively were used. The immunoreactivities were visualized with diaminobenzidine (DAB) (DakoCytomation) and counterstaining was carried out with hematoxylin or Light Green® (Sigma-Aldrich, St. Louis, MO).

2.3. Classification of lung lesions and tumors

Diagnosis of hyperplasia, adenoma and carcinoma in aged B6;129 or 129S4/SvJae mice was carried out according to previously developed criteria [33-35]. Briefly, alveolar Type II cell hyperplasia (also known as bronchiolo-alveolar) was diagnosed by the following features; solitary or multiple, segmental alveolar foci of increased cellularity with the alveolar architecture still detectable, and epithelial cells usually single-layered. Alveolar Type II cell (bronchiolo-alveolar) adenoma was diagnosed by a nodular tumor that was compressing adjacent parenchyma and often less than 3-4 mm in diameter. Alveolar Type II cell (bronchiolo-alveolar) carcinoma was diagnosed by irregular nodular growth, moderately well to poorly circumscribed neoplasms that occupied an entire lobe, composed of papillary connective tissue lined by cuboidal to columnar or pleomorphic cells, with focal areas of tumor cells containing cytoplasm with glycogen and/or neutral lipids. Carcinoma cells also presented increased cellular pleomophism and/or atypia and/or mitotic figures.

In contrast, tumors in CC10TAg mice were of Clara (non-ciliated bronchiolar secretory) cell origin and displayed different morphology from those of the aging mice as previously described [27]. Briefly, by 10 weeks of age, the CC10TAg mice had developed bronchiolar cell hyperplasia associated with marked dysplasia. Normal cuboidal epithelium was replaced by multilayered columnar epithelium composed of highly atypical and dysplastic (transformed) cells with irregular nuclear features, enlarged nuclei and reduced cytoplasm. Multiple tumors of various sizes composed of poorly differentiated cells in sheets, glandular and papillary formation were seen in the alveolar compartment, classified as adenocarcinomas [35]. Type II cells appeared unremarkable.

Human lung carcinomas were diagnosed according to the 1999 WHO International Histological Classification of Tumors [36].

3. Results

3.1. Expression of NKX2-1 and SCGB3A2 in normal mouse lung

Immunohistochemistry was performed to observe expression of NKX2-1 and SCGB3A2 in normal mouse lung. NKX2-1 expression was found in the nuclei of airway epithelial cells and alveolar Type II cells (Fig. 1A, B) while the expression of SCGB3A2 was seen in cytoplasmic and/or apical location of bronchial and bronchiolar epithelial cells (Fig. 1C, D). Normal Type II cells were negative for SCGB3A2 (Fig. 1C, D).

3.2. Neoplastic lesions in the lungs of aging B6;129 and 129S4/SvJae mice

Lung lesions in aging mice with spontaneous tumors [30] consisted of alveolar Type II cell hyperplasia and alveolar Type II cell adenoma and carcinoma (Fig. 2). Lesions were observed in several combinations in the lung of the same mouse as follows; hyperplasia, adenoma or carcinoma only, adenoma and carcinoma together, or all three combined (see Table 1). Hyperplastic epithelia were seen along normal pulmonary alveoli, where NKX2-1 expression was found as seen in normal bronchiolar epithelia (Fig. 2C). The expression of NKX2-1 was also observed in the adenoma cells (Fig. 2G). The level of expression was similar in both non-neoplastic epithelial cells and adenomas (Fig. 3A, B). The NKX2-1 expression was, however, decreased or almost abolished in foci of the carcinomas (Fig. 2K and 3C, D). In contrast to NKX2-1, the expression of SCGB3A2 was not found in either hyperplastic alveolar lesions or adenomas (Fig. 2D, H), while weak to strong SCGB3A2 expression was observed in carcinomas (Fig. 2L). In particular, the expression of SCGB3A2 was strong in the areas where NKX2-1 expression was low or not detected.

3.3. Neoplastic lesions in the lungs of CC10TAg transgenic mice

Pulmonary neoplastic lesions were further examined using a transgenic mouse that expresses SV40 large T antigen under the control of mouse *Scgb1a1* (*CC10*) gene promoter (called CC10TAg mouse) [26]. All mice developed Clara cell adenocarcinomas (Fig. 4). These carcinomas expressed both NKX2-1 and SCGB3A2 (Fig. 4C, G, D, H). In particular, an accumulation of SCGB3A2 was clearly observed in many carcinomas (Fig. 4D). Similar to the spontaneously arisen carcinomas in aging mice, NKX2-1 expression was decreased in the areas where higher level of SCGB3A2 expression was found, or vice versa (Fig. 4C, D, G, H). These results again demonstrated the inverse correlation between NKX2-1 and SCGB3A2 expression. The expression of NKX2-1 and SCGB3A2 in dysplastic airway epithelium (Clara cells) was highly variable, ranging from notably transformed cells with no staining to focally intense expression in other regions without clear correlation in expression patterns between these two genes (Data not shown, Table 1).

3.4. Expression of NKX2-1 and SCGB3A2 in normal human lung

In order to examine the distribution of NKX2-1 and SCGB3A2-containing cells in normal human lungs, we performed immunohistochemistry on specimens obtained from healthy individuals with no evidence of pulmonary cancer or other abnormalities (Fig. 5). Immunoreactivity for NKX2-1 was nuclear and present in the terminal airway (bronchiolar) epithelium and Type II cells throughout the alveolar compartment (Fig. 5A). SCGB3A2 was localized in the cytoplasm or apial portions of bronchiolar epithelial cells, but not in alveolar Type II cells (Fig. 5B). This expression pattern resembles that of normal mouse lung (see Fig. 1). For comparison we performed immunohistochemical staining also for SCGB1A1 (CC10), which demonstrated immunoprecipitation in both cytoplasmic and apical locations

of bronchiolar epithelial cells (Fig. 5C) similar to that seen with SCGB3A2, while Type II cells were negative. The patterns of immunoreactivities in Fig. 5 suggest that at least some of the terminal airway cells co-express all three proteins.

3.5. Expression of NKX2-1 and SCGB3A2 in human NSCLCs and in surrounding lung

In order to correlate expression of NKX2-1 and SCGB3A2 in mouse lung neoplastic lesions to those of humans, a total of 23 human NSCLC specimens (14 adenocarcinomas, 6 squamous cell carcinomas, and 3 large cell carcinomas) were subjected to immunohistochemical analysis for NKX2-1 and SCGB3A2. Out of 23, 17 (11/14 adenocarcinomas, 5/6 squamous cell carcinomas, and 1/3 large cell carcinomas) were positive for SCGB3A2 (70.8%) (Fig. 6). The results were in good agreement with the previous study, in which overall reactivity for SCGB3A2 was observed in 116 of 156 (74.4%) primary lung cancers [29].

SCGB3A2 was expressed in reactive, hyperplastic Type II cells whereas a squamous cell carcinoma in the same section was negative for SCGB3A2 (Fig. 6A). However, some squamous cell carcinomas were positive for SCGB3A2 (Fig. 6B). Papillary adenocarcinomas were strongly positive for both SCGB3A2 (Fig. 6C) and NKX2-1 (Fig. 6D). Expression of both genes was also observed in an atypical adenomatous hyperplasia (AAH; Fig. 6E, F), a premalignant lesion found in NSCLC lung specimens, where it was variable. However, select areas in these sections exhibited high expression of SCGB3A2 in which NKX2-1 expression appeared to be lower or null (see Fig. 6E, F). Further comparisons were difficult, because additional consecutive sections were not available to us. Nevertheless, these results support the previous observation, demonstrating that SCGB3A2 serves as a marker for pulmonary carcinomas, in particular for adenocarcinoma histology in humans [29]. Altogether, the results suggest that SCGB3A2 provides a good marker for pulmonary carcinomas in mice and humans.

4. Discussion

We report in this study that SCGB3A2 provides a useful immunohistochemical marker for pulmonary alveolar and Clara cell carcinomas in mice, and NSCLCs in humans, particularly adenocarcinomas. All mouse carcinomas examined expressed SCGB3A2 while no expression was found in any alveolar Type II cell hyperplasias or in adenomas. On the other hand, expression in Clara cell hyperplasias or dysplasias in mice was variable. Thus, it appears that the expression of SCGB3A2, normally found exclusively in airway epithelial cells, now appeared in lung neoplasms once they had undergone malignant transformation into carcinomas, regardless of the potential cell of origin. Many types of human NSCLC specimens, particularly adenocarcinomas also highly expressed SCGB3A2. These results suggest a novel role for SCGB3A2 as a tumor marker in addition to the ones already known; as a growth factor during fetal lung development [4] and an anti-inflammatory agent in lung inflammation [5].

Genes belonging to the Secretoglobin (SCGB) gene superfamily appear to be grouped into two classes; those having a tumor suppressor function and those being over-expressed in tumors. The first group of genes serving as a tumor suppressor include SCGB1A1 (CC10), the prototypical member of the SCGB gene superfamily [2,3]. In both mice and humans, SCGB1A1 (CC10) is mainly expressed in the airway epithelium [1] while expression of SCGB1A1 (CC10) is minimally detected in tumors derived from the CC10TAg mouse [25,26]. Similarly, less than 10 % of human NSCLCs express SCGB1A1 (CC10) [24]. Further, tumor suppressor activity of SCGB1A1 (CC10) was demonstrated both *in vitro* using NSCLC cell lines transfected with SCGB1A1 expression plasmid [37] and metastatic prostate cell lines treated with recombinant protein [38], and *in vivo* using *Scgb1a1* (*CC10*)-

null mice administered tobacco carcinogen 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) [39].

Another member of SCGB gene superfamily that is known as a tumor suppressor is SCGB3A1, the gene most similar in sequence to SCGB3A2, also known as HIN-1 (high in normal 1) [40] or UGRP2 [41]. Its tumor suppressor function was demonstrated in many human cancers including breast, prostate, lung and pancreatic carcinomas. The tumor suppressor function was due to methylation of the *SCGB3A1* gene promoter, which resulted in loss of SCGB3A1 expression and malignant phenotypes [40,42,43].

The second group of genes in the SCGB gene superfamily that serve as a tumor marker include SCGB2A2 (mammaglobin A) and SCGB1D2 (lipophilin B). Their expression was found in breast and female genital tract cancers such as endometrial, ovarian and cervical cancers [44,45]. Our current study demonstrates that SCGB3A2 belongs to this second group of SCGB family members that is over-expressed in tumors and may serve as a tumor marker. It is interesting that all three genes, SCGB1A1 (CC10), SCGB3A1, and SCGB3A2 are expressed in Clara cells, yet the former two genes serve as a tumor suppressor while the latter may play a role as a tumor marker.

NKX2-1 has been used as a diagnostic marker for primary pulmonary cancers in humans, in particular for adenocarcinomas [18,19,46]. NKX2-1 was recently identified as a lineage-specific oncogene amplified in lung cancer, and the subset of adenocarcinomas that express NKX2-1 required sustained NKX2-1 expression for survival [15-17]. However, no mutations in the *NKX2-1* gene were reported in any adenocarcinomas examined in these studies [15-17]. In the current study in mice, NKX2-1 was expressed in all types of tumors with decreased expression in lung carcinomas. This was somewhat similar to that found in ethyl carbamate-induced lung tumors of *Tgf-β1* heterozygous mice, in which reduced NKX2-1 expression was found in adenomas as compared to high expression in normal lung bronchiolar epithelia. The expression was further decreased in adenocarcinomas as determined by competitive RT-PCR and immunohistochemical analysis [47]. The correlation between the loss of NKX2-1 protein and mRNA expression, and the *NKX2*-1 gene mutation was recently reported in human lung carcinomas [48]. It is possible that the decreased NKX2-1 expression in mouse pulmonary carcinomas may be attributed to a similar mutation of the mouse *Nkx2-1* gene.

The decreased or no expression of NKX2-1 in Type II and Clara cell carcinomas in mice was associated with high levels of SCGB3A2 expression. The similar trend of inverse correlation between SCGB3A2 and NKX2-1 expression was also found in humans although we could not definitely observe this correlation on cell-to-cell basis due to limited availability of serial sections. This is particularly interesting because the expression of SCGB3A2 is normally regulated by NKX2-1[1,6]. Discordant expression of SCGB3A2 and NKX2-1 in neoplastic lesions supports the notion that tumors have aberrant expression of genes and normal transcriptional control may no longer be functional [49]. This could be due to myriad of genetic alterations such as gene mutations and gene amplifications, and/or epigenetic alterations including aberrant DNA methylation and chromatin modifications [49,50]. Other mechanisms may include aberrant RNA splicing [51] or microRNA expression [52].

Gene methylation plays a major role in tissue-specific expression of SCGB3A1 (HIN-1) [53] and as a tumor suppressor in a majority of breast tumors [43]. It is also possible that transcription factors that play a role in the expression of SCGB3A2 in normal tissues may have different expression patterns in tumors. C/EBPα and C/EBPδ are among those transcription factors regulating SCGB3A2 expression in synergistic interaction with

NKX2-1 [32]. In normal lungs, C/EBPα, C/EBPδ and NKX2-1 are all expressed in airway epithelial and Type II cells albeit to different degrees, and temporal and spacial combination of their expression may influence the pattern of SCGB3A2 expression [32]. The mechanistic reason for the inverse correlation between SCGB3A2 and NKX2-1 expression in carcinomas is not currently understood. Further studies are required to address this question.

In conclusion, SCGB3A2 may be a useful marker for diagnosis of pulmonary tumors in both mice and humans, and may indicate important roles for this gene in pulmonary carcinogenesis.

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References

1. Niimi T, Keck-Waggoner CL, Popescu NC, Zhou Y, Levitt RC, Kimura S. UGRP1, a uteroglobin/Clara cell secretory protein-related protein, is a novel lung-enriched downstream target gene for the T/EBP/NKX2. 1 homeodomain transcription factor Mol Endocrinol 2001;15:2021–2036.
2. Klug J, Beier HM, Bernard A, Chilton BS, Fleming TP, Lehrer RI, Miele L, Pattabiraman N, Singh G. Uteroglobin/Clara cell 10-kDa family of proteins: nomenclature committee report. Ann N Y Acad Sci 2000;923:348–354. [PubMed: 11193777]
3. Mukherjee AB, Kundu GC, Mantile-Selvaggi G, Yuan CJ, Mandal AK, Chattopadhyay S, Zheng F, Pattabiraman N, Zhang Z. Uteroglobin: a novel cytokine? Cell Mol Life Sci 1999;55:771–787. [PubMed: 10379362]
4. Kurotani R, Tomita T, Yang Q, Carlson BA, Chen C, Kimura S. Role of secretoglobin 3A2 in lung development. Am J Respir Crit Care Med 2008;178:389–398. [PubMed: 18535256]
5. Chiba Y, Kurotani R, Kusakabe T, Miura T, Link BW, Misawa M, Kimura S. Uteroglobin-related protein 1 expression suppresses allergic airway inflammation in mice. Am J Respir Crit Care Med 2006;173:958–964. [PubMed: 16456148]
6. Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes & Dev 1996;10:60–69. [PubMed: 8557195]
7. Bruno MD, Bohinski RJ, Huelsman KM, Whitsett JA, Korfhagen TR. Lung cell-specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. J Biol Chem 1995;270:6531–6536. [PubMed: 7896788]
8. Bohinski RJ, Di Lauro R, Whitsett JA. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. Mol Cell Biol 1994;14:5671–5681. [PubMed: 8065304]
9. Kelly SE, Bachurski CJ, Burhans MS, Glasser SW. Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. J Biol Chem 1996;271:6881–6888. [PubMed: 8636114]
10. Ray MK, Chen CY, Schwartz RJ, DeMayo FJ. Transcriptional regulation of a mouse Clara cell-specific protein (mCC10) gene by the NKx transcription factor family members thyroid transciption factor 1 and cardiac muscle-specific homeobox protein (CSX). Mol Cell Biol 1996;16:2056–2064. [PubMed: 8628271]
11. Poornima S, Christian C, Kresch MJ. Developmental regulation of SP-A receptor in fetal rat lung. Lung 2002;180:33–46. [PubMed: 12105755]
12. Strayer M, Savani RC, Gonzales LW, Zaman A, Cui Z, Veszelovszky E, Wood E, Ho YS, Ballard PL. Human surfactant protein B promoter in transgenic mice: temporal, spatial, and stimulus-responsive regulation. Am J Physiol Lung Cell Mol Physiol 2002;282:L394–404. [PubMed: 11839532]

13. Glasser SW, Eszterhas SK, Detmer EA, Maxfield MD, Korfhagen TR. The murine SP-C promoter directs type II cell-specific expression in transgenic mice. Am J Physiol Lung Cell Mol Physiol 2005;288:L625–632. [PubMed: 15579627]

14. Xu P, Hashimoto S, Miyazaki H, Asabe K, Shiraishi S, Sueishi K. Morphometric analysis of the immunohistochemical expression of Clara cell 10-kDa protein and surfactant apoproteins A and B in the developing bronchi and bronchioles of human fetuses and neonates. Virchows Arch 1998;432:17–25. [PubMed: 9463583]

15. Kwei KA, Kim YH, Girard L, Kao J, Pacyna-Gengelbach M, Salari K, Lee J, Choi YL, Sato M, Wang P, Hernandez-Boussard T, Gazdar AF, Petersen I, Minna JD, Pollack JR. Genomic profiling identifies TITF1 as a lineage-specific oncogene amplified in lung cancer. Oncogene 2008;27:3635–3640. [PubMed: 18212743]

16. Tanaka H, Yanagisawa K, Shinjo K, Taguchi A, Maeno K, Tomida S, Shimada Y, Osada H, Kosaka T, Matsubara H, Mitsudomi T, Sekido Y, Tanimoto M, Yatabe Y, Takahashi T. Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1. Cancer Res 2007;67:6007–6011. [PubMed: 17616654]

17. Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, Lin WM, Province MA, Kraja A, Johnson LA, Shah K, Sato M, Thomas RK, Barletta JA, Borecki IB, Broderick S, Chang AC, Chiang DY, Chirieac LR, Cho J, Fujii Y, Gazdar AF, Giordano T, Greulich H, Hanna M, Johnson BE, Kris MG, Lash A, Lin L, Lindeman N, Mardis ER, McPherson JD, Minna JD, Morgan MB, Nadel M, Orringer MB, Osborne JR, Ozenberger B, Ramos AH, Robinson J, Roth JA, Rusch V, Sasaki H, Shepherd F, Sougnez C, Spitz MR, Tsao MS, Twomey D, Verhaak RG, Weinstock GM, Wheeler DA, Winckler W, Yoshizawa A, Yu S, Zakowski MF, Zhang Q, Beer DG, Wistuba II, Watson MA, Garraway LA, Ladanyi M, Travis WD, Pao W, Rubin MA, Gabriel SB, Gibbs RA, Varmus HE, Wilson RK, Lander ES, Meyerson M. Characterizing the cancer genome in lung adenocarcinoma. Nature 2007;450:893–898. [PubMed: 17982442]

18. Di Loreto C, Di Lauro V, Puglisi F, Damante G, Fabbro D, Beltrami CA. Immunocytochemical expression of tissue specific transcription factor-1 in lung carcinoma. J Clin Pathol 1997;50:30–32. [PubMed: 9059352]

19. Nakamura N, Miyagi E, Murata S, Kawaoi A, Katoh R. Expression of thyroid transcription factor-1 in normal and neoplastic lung tissues. Mod Pathol 2002;15:1058–1067. [PubMed: 12379752]

20. Camilo R, Capelozzi VL, Siqueira SA, Del Carlo Bernardi F. Expression of p63, keratin 5/6, keratin 7, and surfactant-A in non-small cell lung carcinomas. Hum Pathol 2006;37:542–546. [PubMed: 16647951]

21. Bakir K, Kocer NE, Deniz H, Guldur ME. TTF-1 and surfactant-B as co-adjuvants in the diagnosis of lung adenocarcinoma and pleural mesothelioma. Ann Diagn Pathol 2004;8:337–341. [PubMed: 15614737]

22. Burke LM, Rush WI, Khoor A, Mackay B, Oliveira P, Whitsett JA, Singh G, Turnicky R, Fleming MV, Koss MN, Travis WD. Alveolar adenoma: a histochemical, immunohistochemical, and ultrastructural analysis of 17 cases. Hum Pathol 1999;30:158–167. [PubMed: 10029443]

23. Yamamoto O, Takahashi H, Hirasawa M, Chiba H, Shiratori M, Kuroki Y, Abe S. Surfactant protein gene expressions for detection of lung carcinoma cells in peripheral blood. Respir Med 2005;99:1164–1174. [PubMed: 16085219]

24. Linnoila RI, Szabo E, DeMayo F, Witschi H, Sabourin C, Malkinson A. The role of CC10 in pulmonary carcinogenesis: from a marker to tumor suppression. Ann N Y Acad Sci 2000;923:249–267. [PubMed: 11193761]

25. Hicks SM, Vassallo JD, Dieter MZ, Lewis CL, Whiteley LO, Fix AS, Lehman-McKeeman LD. Immunohistochemical analysis of Clara cell secretory protein expression in a transgenic model of mouse lung carcinogenesis. Toxicology 2003;187:217–228. [PubMed: 12699910]

26. Magdaleno SM, Wang G, Mireles VL, Ray MK, Finegold MJ, DeMayo FJ. Cyclin-dependent kinase inhibitor expression in pulmonary Clara cells transformed with SV40 large T antigen in transgenic mice. Cell Growth Differ 1997;8:145–155. [PubMed: 9040936]

27. Linnoila RI, Sahu A, Miki M, Ball DW, DeMayo FJ. Morphometric analysis of CC10-hASH1 transgenic mouse lung: a model for bronchiolization of alveoli and neuroendocrine carcinoma. Exp Lung Res 2000;26:595–615. [PubMed: 11195458]

28. Linnoila RI, Zhao B, DeMayo JL, Nelkin BD, Baylin SB, DeMayo FJ, Ball DW. Constitutive achaete-scute homologue-1 promotes airway dysplasia and lung neuroendocrine tumors in transgenic mice. Cancer Res 2000;60:4005–4009. [PubMed: 10945598]
29. Tachihara-Yoshikawa M, Ishida T, Watanabe K, Sugawawa A, Kanazawa K, Kanno R, Suzuki T, Niimi T, Kimura S, Munakata M. Expression of secretoglobin3A2 (SCGB3A2) in primary pulmonary carcinomas. Fukushima J Med Sci 2008;54:61–72. [PubMed: 19418968]
30. Haines DC, Chattopadhyay S, Ward JM. Pathology of aging B6;129 mice. Toxicol Pathol 2001;29:653–661. [PubMed: 11794381]
31. Ward, JM.; Anver, MR.; Mahler, JF.; Devor-Henneman, DE. Pathology of Mice Commonly Used in Genetic Engineering (C57BL/6; 129; B6, 129 and FVB/N). Ames: Iowa State University Press; 2000.
32. Tomita T, Kido T, Kurotani R, Iemura S, Sterneck E, Natsume T, Vinson C, Kimura S. CAATT/Enhancer-binding Proteins {alpha} and {delta} Interact with NKX2-1 to Synergistically Activate Mouse Secretoglobin 3A2 Gene Expression. J Biol Chem 2008;283:25617–25627. [PubMed: 18632661]
33. Mohr, U., editor. WHO International Agency for Research on Cancer. International Classification of Rodent Tumors The mouse. Berlin, New York: Springer; 2001.
34. Ward JM, Singh G, Katyal SL, Anderson LM, Kovatch RM. Immunocytochemical localization of the surfactant apoprotein and Clara cell antigen in chemically induced and naturally occurring pulmonary neoplasms of mice. Am J Pathol 1985;118:493–499. [PubMed: 3883798]
35. Nikitin AY, Alcaraz A, Anver MR, Bronson RT, Cardiff RD, Dixon D, Fraire AE, Gabrielson EW, Gunning WT, Haines DC, Kaufman MH, Linnoila RI, Maronpot RR, Rabson AS, Reddick RL, Rehm S, Rozengurt N, Schuller HM, Shmidt EN, Travis WD, Ward JM, Jacks T. Classification of proliferative pulmonary lesions of the mouse: recommendations of the mouse models of human cancers consortium. Cancer Res 2004;64:2307–2316. [PubMed: 15059877]
36. Travis, WD.; Colby, TV.; Corrin, B.; Shimosato, Y.; Brambilla, E., editors. WHO International Classification of tumors. New York: Springer; 1999. Histological typing of lung and pleural tumours.
37. Szabo E, Goheer A, Witschi H, Linnoila RI. Overexpression of CC10 modifies neoplastic potential in lung cancer cells. Cell Growth Differ 1998;9:475–485. [PubMed: 9663466]
38. Leyton J, Manyak MJ, Mukherjee AB, Miele L, Mantile G, Patierno SR. Recombinant human uteroglobin inhibits the in vitro invasiveness of human metastatic prostate tumor cells and the release of arachidonic acid stimulated by fibroblast-conditioned medium. Cancer Res 1994;54:3696–3699. [PubMed: 8033085]
39. Yang Y, Zhang Z, Mukherjee AB, Linnoila RI. Increased susceptibility of mice lacking Clara cell 10-kDa protein to lung tumorigenesis by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a potent carcinogen in cigarette smoke. J Biol Chem 2004;279:29336–29340. [PubMed: 15148323]
40. Krop I, Parker MT, Bloushtain-Qimron N, Porter D, Gelman R, Sasaki H, Maurer M, Terry MB, Parsons R, Polyak K. HIN-1, an inhibitor of cell growth, invasion, and AKT activation. Cancer Res 2005;65:9659–9669. [PubMed: 16266985]
41. Niimi T, Copeland NG, Gilbert DJ, Jenkins NA, Srisodsai A, Zimonjic DB, Keck-Waggoner CL, Popescu NC, Kimura S. Cloning, expression, and chromosomal localization of the mouse gene (Scgb3a1, alias Ugrp2) that encodes a member of the novel uteroglobin-related protein gene family. Cytogenet Genome Res 2002;97:120–127. [PubMed: 12438750]
42. Krop IE, Sgroi D, Porter DA, Lunetta KL, LeVangie R, Seth P, Kaelin CM, Rhei E, Bosenberg M, Schnitt S, Marks JR, Pagon Z, Belina D, Razumovic J, Polyak K. HIN-1, a putative cytokine highly expressed in normal but not cancerous mammary epithelial cells. Proc Natl Acad Sci U S A 2001;98:9796–9801. [PubMed: 11481438]
43. Krop I, Player A, Tablante A, Taylor-Parker M, Lahti-Domenici J, Fukuoka J, Batra SK, Papadopoulos N, Richards WG, Sugarbaker DJ, Wright RL, Shim J, Stamey TA, Sellers WR, Loda M, Meyerson M, Hruban R, Jen J, Polyak K. Frequent HIN-1 promoter methylation and lack of expression in multiple human tumor types. Mol Cancer Res 2004;2:489–494. [PubMed: 15383627]

44. Watson MA, Dintzis S, Darrow CM, Voss LE, DiPersio J, Jensen R, Fleming TP. Mammaglobin expression in primary, metastatic, and occult breast cancer. Cancer Res 1999;59:3028–3031. [PubMed: 10397237]
45. Zafrakas M, Petschke B, Donner A, Fritzsche F, Kristiansen G, Knuchel R, Dahl E. Expression analysis of mammaglobin A (SCGB2A2) and lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. BMC Cancer 2006;6:88. [PubMed: 16603086]
46. Barlesi F, Pinot D, Legoffic A, Doddoli C, Chetaille B, Torre JP, Astoul P. Positive thyroid transcription factor 1 staining strongly correlates with survival of patients with adenocarcinoma of the lung. Br J Cancer 2005;93:450–452. [PubMed: 16052216]
47. Kang Y, Hebron H, Ozbun L, Mariano J, Minoo P, Jakowlew SB. Nkx2.1 transcription factor in lung cells and a transforming growth factor-beta1 heterozygous mouse model of lung carcinogenesis. Mol Carcinog 2004;40:212–231. [PubMed: 15264213]
48. Bai XY, Shen H. Mutational analysis of thyroid transcription factor-1 gene (TTF-1) in lung carcinomas. In Vitro Cell Dev Biol Anim 2008;44:17–25. [PubMed: 18071837]
49. Ellis L, Atadja PW, Johnstone RW. Epigenetics in cancer: targeting chromatin modifications. Mol Cancer Ther 2009;8:1409–1420. [PubMed: 19509247]
50. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135:1079–1099. [PubMed: 18773902]
51. Fackenthal JD, Godley LA. Aberrant RNA splicing and its functional consequences in cancer cells. Dis Model Mech 2008;1:37–42. [PubMed: 19048051]
52. Papagiannakopoulos T, Kosik KS. MicroRNAs: regulators of oncogenesis and stemness. BMC Med 2008;6:15. [PubMed: 18577221]
53. Tomita T, Kimura S. Regulation of mouse Scgb3a1 gene expression by NF-Y and association of CpG methylation with its tissue-specific expression. BMC Mol Biol 2008;9:5. [PubMed: 18194566]

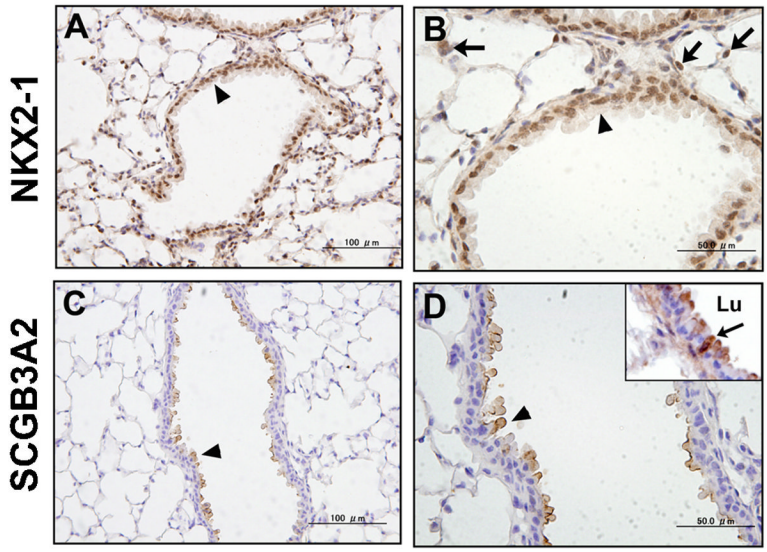

Fig. 1.

NKX2-1 and SCGB3A2 expression in normal mouse pulmonary epithelia.

(A, B) NKX2-1 imunohistochemistry, (C, D) SCGB3A2 immunohistochemistry. B and D (×400) are magnified pictures of A and C (×200). Brown color indicates positive staining. NKX2-1 was detected in nuclei of bronchiolar epithelial cells (A, B, shown by arrowhead) and Type II cells (B, representatives shown by arrows) while SCGB3A2 was detected in the luminal borders of bronchiolar epithelial cells (C, D, shown by arrowhead). SCGB3A2 staining can be seen at apical (arrowhead in D) or cytoplasmic (insert in D) locations. Lu=bronchiolar lumen.

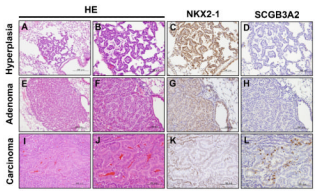

Fig. 2.

Histology of pulmonary tumorigenesis in aging mice.

(A, B, E, F, I, J) H&E staining. (C, G, K) immunohistochemistry for NKX2-1 and (D, H, L) for SCGB3A2. (A-D) hyperplasia, (E-H) adenoma, (I-L) carcinoma, found in aging mice. Sections were serially prepared. B, F, and J (×400) are magnified pictures of A, E, and I (×200), respectively. Brown color indicates positive staining. Strong NKX2-1 signals were observed in hyperplastic epithelial cells (C), and the weak signal was partially observed in carcinomas (K). Strong SCGB3A2 was expressed in the areas where NKX2-1 expression was reduced or almost abolished (L).

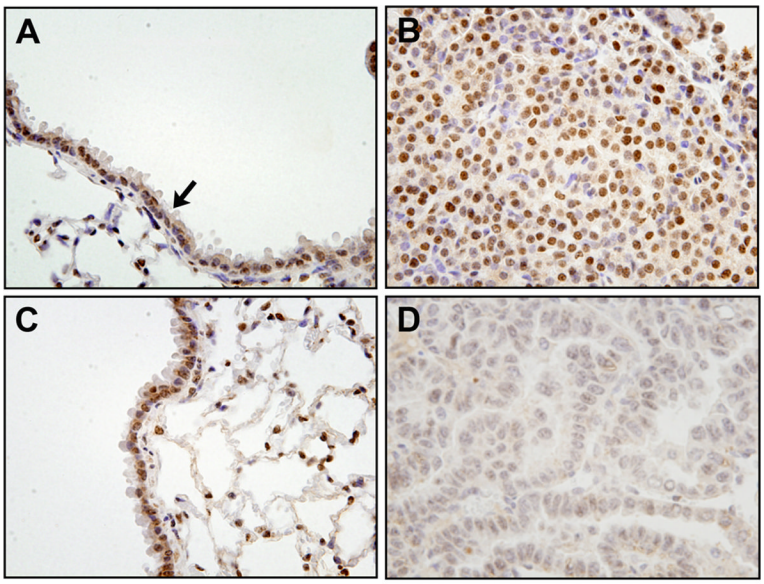

Fig. 3.

NKX2-1 expression in pulmonary alveolar Type II cell adenoma and carcinoma of B6;129 aging mice.

(A) Non-neoplastic bronchiolar epithelial cells, (B) alveolar Type II cell adenoma cells, (C) non-neoplastic normal bronchiolar epithelial cells in lung with a carcinoma, (D) a portion of a carcinoma. Brown color indicates positive staining. NKX2-1 immunohistochemical signal in adenoma (B) was as intense as that in non-neoplastic bronchiolar epithelial cells (A, shown by an arrow), while expression was absent focally in carcinoma (D).

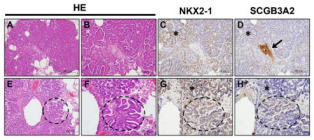

Fig. 4.

Histopathology of adenocarcinoma in CC10TAg mouse lung.

(A, B, E, F) H&E staining. B and F (×400) are high magnifications of A and E (×200), respectively. (C, G) Immunohistochemistry for NKX2-1, and (D, H) for SCGB3A2. Sections were serially prepared. Brown color indicates positive staining. Some areas of carcinoma in which NKX2-1 was expressed did not express SCGB3A2 (C, D, G, H, asterisks), while the area of carcinoma in which NKX2-1 expression was decreased (G, broken circle) expressed SCGB3A2 (H, broken circle). Arrow in D indicates strong SCGB3A2 signal that appears to be an accumulation of protein due to exudation, production and/or cellular release of SCGB3A2.

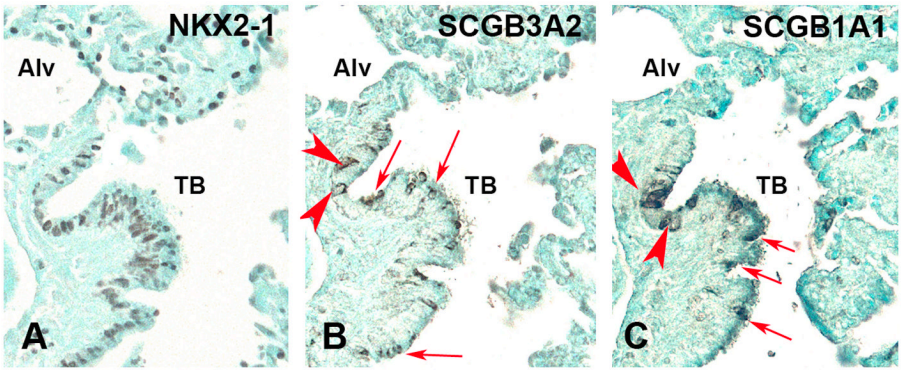

Fig. 5.

Expression of NKX2-1 and secretoglobins in human terminal respiratory units.

(A) NKX2-1 was expressed in both alveolar Type II cells as well as in the epithelium of terminal bronchioles. Immunoreactivity was nuclear. (B) Both cytoplasmic (red arrowheads) and apical (red arrows) pattern was seen in SCGB3A2-containing terminal bronchiolar cells, while Type II cells remained negative. (C) Bronchiolar epithelial cells were positive for SCGB1A1 (CC10) and Type II cells showed no staining. Both cytoplasmic (red arrow heads) and apical (red arrows) staining was observed. Alv=alveoli; TB=terminal bronchiolus where immunoperoxidase stain is seen. Original magnification, ×200.

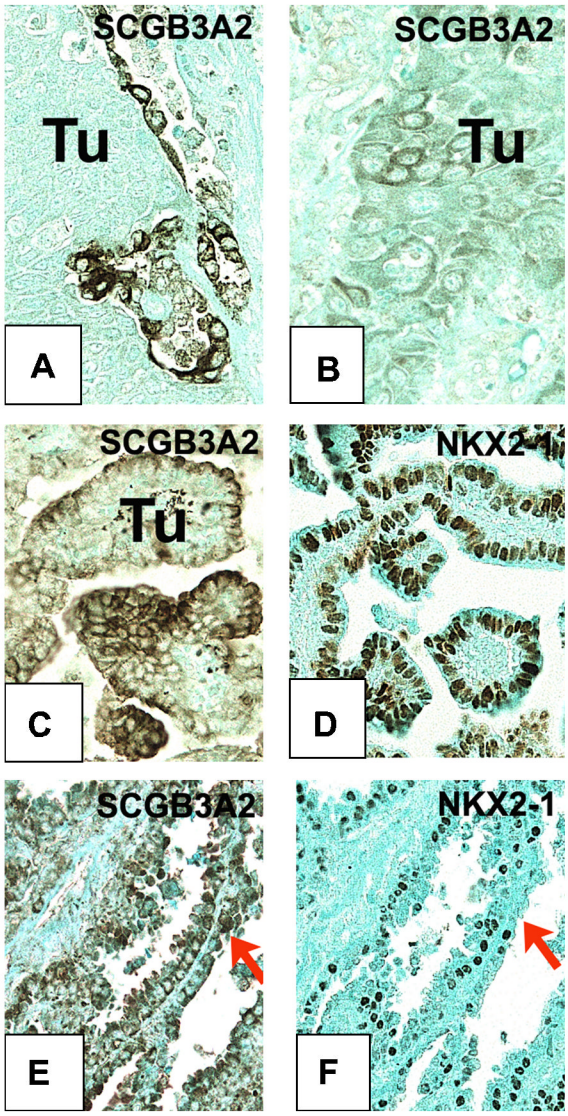

Fig. 6.

SCGB3A2 and NKX2-1 expression in human NSCLCs.

(A, B, C, E) Immunohistochemistry for SCGB3A2, and (D, F) for NKX2-1. Representative results are shown. (A) SCGB3A2-positive hyperplastic Type II cells adjacent to a negative squamous cell carcinoma (shown by Tu: tumor). (B) SCGB3A2-positive tumor cells in a squamous cell carcinoma. Strongly positive for both NKX2-1 and SCGB3A2 in (C, D) papillary adenocarcinoma and (E, F) atypical adenomatous hyperplasia (AAH). Red arrows in E and F show the area where the level of SCGB3A2 and NKX2-1 expression may be inversely correlated. Original magnification for all panels ×200.

Table 1

Expression of NKX2-1 and SCGB3A2 in pulmonary preneoplastic and neoplastic lesions

	NKX2-1	%	SCGB3A2	%
Alveolar Type II Hyperplasia[1]	12/12	100	0/12	0
Alveolar Type II Adenoma[1]	18/18	100	0/18[3]	0
Alveolar Type II Carcinoma[1]	13/13	100	13/13	100
Bronchiolar Clara Cell Hyper/Dysplasia[2]	9/9	100	9/9[4]	100
Clara cell adenocarcinoma[2]	9/9	100	9/9	100

[1] Lesions found in a total of 28 aging B6;129 and 129S4/SvJae mice. Total number of tumors was 31.

[2] Lesions found in nine 10-16 weeks-old CC10TAg mice.

[3] Two of eighteen adenomas expressed less than 1% of SCGB3A2.

[4] Expression ranged from negative to intense.