Gene Expression Profiling Revealed 2 Types of Bronchial Basal Cell Hyperplasia and Squamous Metaplasia With Different Progression Potentials

Evgeny V. Denisov, PhD,† Anastasia A. Schegoleva, MSc,**
Tatiana S. Gerashchenko, MD, PhD,† Nikolay A. Skryabin, MD, PhD,**
Alexey A. Sleptcov, MD, PhD, Valentina D. Yakushina, MD, PhD,‡ Liliya S. Lyapunova, MD,**
Sergey A. Tuzikov, MD, PhD, Olga V. Pankova, MD, PhD,**
*and Vladimir M. Perelmuter, MD, PhD**

Abstract: The premalignant process preceding squamous cell lung cancer is not inevitable; it can stop at any of the bronchial lesions: basal cell hyperplasia (BCH), squamous metaplasia (SM), and dysplasia and then progress or regress. At present, the mechanisms underlying the progression of the bronchial lesions remain undefined. Previously, we hypothesized that bronchial lesions that presented individually or combined with each other in the bronchi of lung cancer patients mirror the different "scenarios" of the premalignant process: individual BCH—the stoppage at the stage of hyperplasia, BCH plus SM—the progression of hyperplasia to metaplasia, and SM plus dysplasia—the progression of metaplasia to dysplasia. In this study, we analyzed gene expression profiles of BCH, SM, and dysplasia depending on their cooccurrence in the bronchi of lung cancer patients. The immune response gene expression was found to be a key difference between the individual BCH and BCH combined with SM lesions and a potential mechanism that determines the progression of hyperplasia to metaplasia. Upregulation of the cell cycle and downregulation of the cilium assembly genes mainly distinguished SM that copresented with dysplasia from SM that copresented with BCH and is a probable mechanism of the progression of metaplasia to dysplasia. Dysplasia showed mainly overexpression of the cell division genes and underexpression of the inflammation genes. Thus, this study demonstrates the significant gene expression differences between the premalignant lesions depending on their cooccurrence in the bronchi and sheds light on the mechanisms of the precancerous process preceding squamous cell lung cancer.

Key Words: lung cancer, basal cell hyperplasia, squamous metaplasia, dysplasia, gene expression

(*Appl Immunohistochem Mol Morphol* 2019;00:000–000)

Lung cancer has the highest incidence among all kinds of malignant tumors and is the most common cause of cancer-related death worldwide. Despite the progress in diagnosis and treatment, lung cancer continues to be detected at late stages. In this aspect, it seems relevant to shift the focus on the precancerous (premalignant) process in the bronchial epithelium in order to prevent the development of lung cancer.

Squamous cell lung carcinoma, one of the most common forms of non–small cell lung cancer (NSCLC), shows an aggressive behavior and is frequently detected at an advanced inoperable stage. Squamous cell lung carcinoma is thought to arise from premalignant lesions in the airway epithelium: basal cell hyperplasia (BCH), squamous metaplasia (SM), and dysplasia.[1,2] The precancerous process can be both sequential (BCH—SM—dysplasia) and discontinuous when it can stop at any of the bronchial lesions, then either progress or regress. Many studies described the possible role of genetic and epigenetic alterations, and gene and protein expression changes in the premalignant process in the bronchial epithelium.[2–5] However, the mechanisms underlying the progression or the regression of bronchial changes remain poorly understood. In addition, no effective markers have been developed to predict the risk of bronchial lesion progression.

The premalignant changes are frequently seen in the bronchial epithelium of lung cancer patients and probably represent the independent precancerous processes. The first observations were made in the 1930-1950s and affected the main bronchi distant from the primary tumor.[6] Subsequent works focused on the bronchial lesions accompanying lung tumors to investigate the molecular mechanisms involved in the precancerous process.[5,7–9] In our study, we found that the bronchial lesions also occurred in the small bronchi of

Received for publication January 18, 2019; accepted February 25, 2019.
From the *Tomsk National Research Medical Center; †Laboratory for Translational Cellular and Molecular Biomedicine, Tomsk State University, Tomsk; and ‡Research Centre for Medical Genetics, Moscow, Russia.
Supported by the Russian Foundation for Basic Research (No. 17-29-06002).
The authors declare no conflict of interest.
Reprints: Evgeny V. Denisov, PhD, Tomsk National Research Medical Center, Kooperativny Str. 5, Tomsk 634009, Russia (e-mail: d_evgeniy@oncology.tomsk.ru).
Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.appliedimmunohist.com.

NSCLC patients and can be detected in various combinations with each other: individual (isolated) BCH, BCH plus SM, and SM plus dysplasia.[10] These combinations were hypothesized to reflect the different "scenarios" of the precancerous process: individual BCH (iBCH)—the stoppage at the stage of hyperplasia, BCH plus SM—the progression of hyperplasia to metaplasia, and SM plus dysplasia—the progression of metaplasia to dysplasia (oncogenic phase[11]). This assumption is supported by the fact that isolated and copresented forms of BCH and SM are recurrently observed in NSCLC patients, show the specific immunohistochemical profile, and have a high clinical significance.[12] Thus, the different combinations of the bronchial lesions can be an attractive model to explore the mechanisms underlying the premalignant process.

In this study, we aimed to assess gene expression profiles of BCH, SM, and dysplasia depending on their cooccurrence in the bronchi of NSCLC patients and to identify biological processes that are involved in the different scenarios of the premalignant process in the respiratory epithelium.

MATERIALS AND METHODS

The samples of lung tissue (small bronchi) were obtained at a distance of 4 to 5 cm from the tumor during surgery of 15 NSCLC patients. The histologic diagnosis of lung cancer was made according to the IASLC/ATS/ERS lung adenocarcinoma classification[13] and WHO criteria.[14] The identification of BCH, SM, and dysplasia in the bronchial epithelium (Fig. 1A) was carried out using standard recommendations.[1,15] All cases were without preoperative chemotherapy. The characteristics of NSCLC patients and the distribution of the bronchial lesions are shown in Table 1.

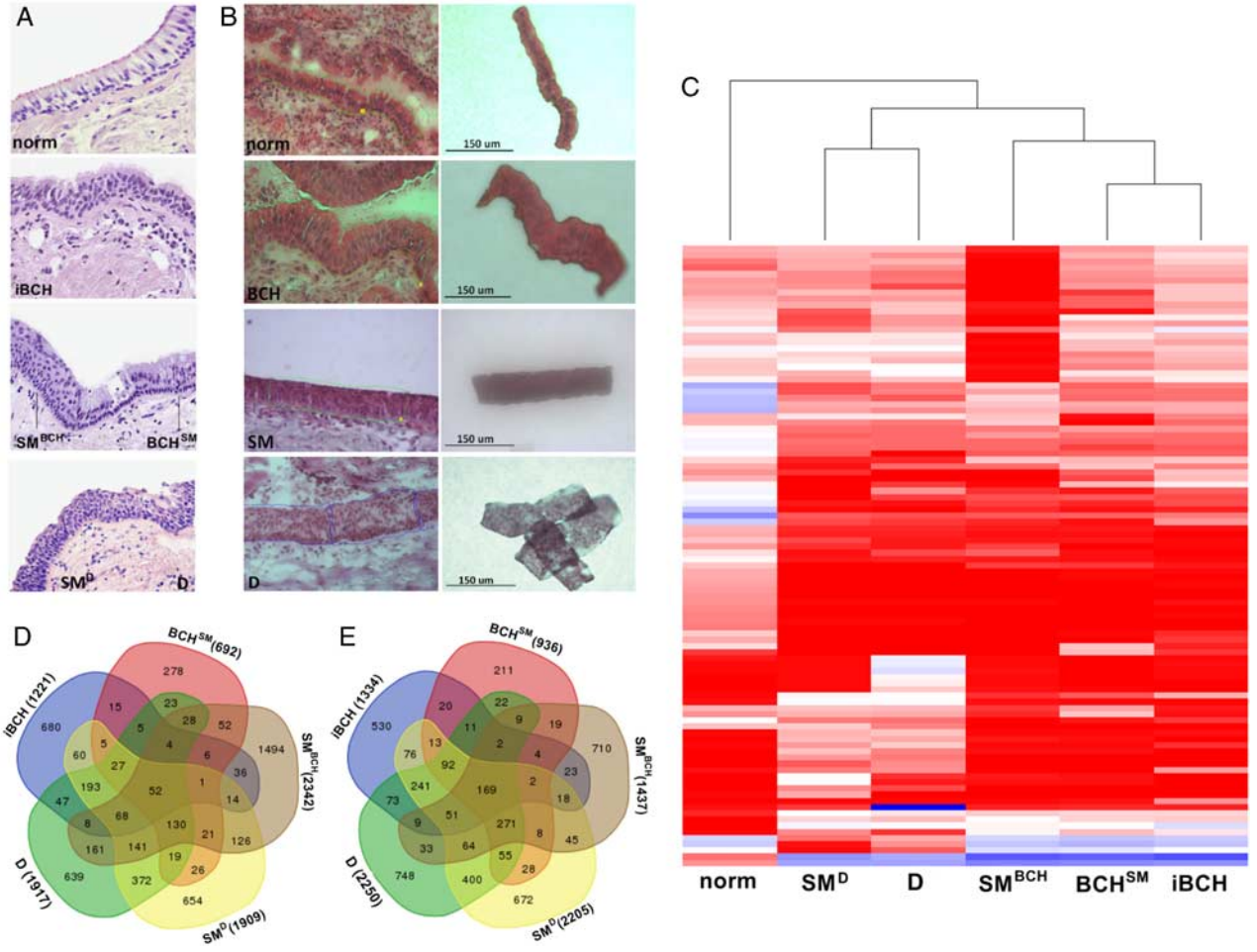

FIGURE 1. Gene expression profile of the bronchial lesions. A, The normal bronchial epithelium and the lesions presented individually and in combination with each other. B, Laser microdissection of the bronchial lesions. C, Heat map and unsupervised hierarchical clustering analysis of the 100 most significantly dysregulated genes in the bronchial lesions compared with the normal breast epithelium. D and E, Venn diagrams of upregulated and downregulated genes in the bronchial lesions. BCH^{SM} indicates basal cell hyperplasia that copresented with squamous metaplasia; iBCH, individual basal cell hyperplasia; Norm, normal bronchial epithelium; SM^{BCH}, squamous metaplasia that copresented with basal cell hyperplasia; SM^{D}, squamous metaplasia that copresented with dysplasia.

The fresh frozen samples obtained from 6 patients were used for laser microdissection and gene expression microarray analysis. The formalin-fixed paraffin-embedded samples of 9 patients were used to perform immunohistochemical staining.

The procedures followed in this study were in accordance with the Helsinki Declaration (1964, amended in 1975 and 1983). All patients signed an informed consent for voluntary participation. The study was approved by the institutional review board of the Tomsk Cancer Research Institute on the 10th of December 2012 (the approval number is 16).

Laser Microdissection

The normal bronchial epithelium and the premalignant lesions were obtained from hematoxylin and eosin–stained 5-μm-thick sections of lung tissue samples using laser microdissection PALM (Carl Zeiss, Germany; Fig. 1B). Up to 50 microdissected samples of the normal epithelium and each bronchial lesion were collected. In general, the following samples were microdissected:
(1) The normal bronchial epithelium (n = 1).
(2) iBCH (n = 2).
(3) BCH that copresented with SM (BCH^{SM}; n = 2).
(4) SM that copresented with BCH (SM^{BCH}; n = 2).
(5) SM that copresented with dysplasia (SM^{D}; n = 1).
(6) Dysplasia (n = 1).

RNA Isolation and Whole-Transcriptome Amplification

Total RNA was extracted from the microdissected samples using the RNeasy Plus Micro Kit (Qiagen, USA). RNA integrity varied from 4.6 to 7.9. RNA samples were amplified using the Ovation PicoSL WTA System V2 kit (NuGEN, USA). Amplified products (cDNA) were purified using the QIAquick PCR Purification Kit (Qiagen).

Gene Expression Microarrays

The cDNA samples were labeled with Cy3 fluorescent dye (SureTag DNA labeling kit, Agilent, USA) and hybridized on the SurePrint G3 Human GE v2 8×60 K microarrays (Agilent). Hybridization, subsequent washing, and drying of the slides were performed according to the Agilent instructions with the following modifications: 2 μg of the labeled cDNA was hybridized for 22 hours at 65°C, and the cDNA was not fragmented before hybridization. The scanning was carried out using a SureScan Microarray Scanner, and signals were extracted using Feature Extraction software version 10.7.3.1 (Agilent). The microarray data are available under GEO accession number GSE80754.

Immunohistochemical Staining

The specific expression of 3 genes (*CCDC114*, *MAP7D2*, and *LIFR*) was confirmed in the premalignant lesions using immunohistochemical staining (IHC), as previously described.[16] The following antibodies were used: CCDC114 (HPA042524, 1:1000, Sigma, USA), MAP7D2 (HPA051508, 1:100, Sigma), and LIFR (GTX60183, 1:500, GeneTex, USA). The percentage of stained cells with any degree of protein expression was assessed in different parts of the tissue sections (1000 cells in 10 fields of view) corresponding to the BCH, SM, and dysplasia.

Bioinformatic, Statistical, and Functional Enrichment Analyses

The gene expression microarray data were evaluated using the R software (R Development Core Team, 2008) and the Limma package from BioConductor.[17] Log mean spot signals were taken for further analysis. Expression levels were normalized to the normal bronchial epithelium. Genes were ranked for differential expression using a moderated *t*-statistic, as implemented in the Limma package. The identification of shared and specific genes was carried out by Venn diagram analysis using Draw Venn (http://bioinformatics.psb.ugent.be/webtools/Venn/). Upregulated and downregulated genes with |log-fold-change| $\geq \log_2 1.5$ and an unadjusted *P*-value <0.05 were used in GO enrichment analysis.[18] A false discovery

TABLE 1. Clinicopathologic Characteristics of Lung Cancer Patients

Cases	Age (y)	Tumor Localization	Histologic Type	TNM	Bronchial Lesions	Analysis
1	55	Peripheral, left middle lobe	Squamous cell carcinoma	T3N1M0	BCH+SM	Gene expression microarrays
2	48	Central, right middle lobe	Squamous cell carcinoma	T3N0M0	SM+D	
3	67	Central, right lower lobe	Squamous cell carcinoma	T3N0M0	NE	
4	61	Peripheral, left upper lobe	Adenocarcinoma	T3N0M0	iBCH	
5	59	Peripheral, right middle lobe	Adenocarcinoma	T2N0M0	BCH+SM	
6	58	Central, left upper lobe	Squamous cell carcinoma	T3N0M0	iBCH	
7	53	Central, right lower lobe	Squamous cell carcinoma	T3N2M0	iBCH	IHC
8	76	Central, right upper lobe	Adenocarcinoma	T3N0M0	iBCH	
9	67	Central, right middle lobe	Squamous cell carcinoma	T3N1M0	iBCH	
10	58	Central, left lower lobe	Squamous cell carcinoma	T2N0M0	BCH+SM	
11	70	Peripheral, left upper lobe	Squamous cell carcinoma	T3N0M0	BCH+SM	
12	62	Peripheral, right upper lobe	Adenocarcinoma	T1N0M0	BCH+SM	
13	64	Central, left upper lobe	Adenocarcinoma	T3N0M0	BCH+SM	
14	63	Peripheral, left lower lobe	Squamous cell carcinoma	T3N0M0	BCH+SM	
15	50	Central, right upper lobe	Squamous cell carcinoma	T3N0M0	SM+D	

BCH+SM indicates the copresence of basal cell hyperplasia and squamous metaplasia; iBCH, individual basal cell hyperplasia; IHC, immunohistochemistry; NE, normal epithelium; SM+D, the co-presence of squamous metaplasia and dysplasia; TNM, tumor-node-metastasis classification.

rate (FDR) < 0.05 was used as a threshold to consider biological processes as being significantly enriched.

RESULTS

Gene expression profiling showed that BCH, SM, and dysplasia differ from the normal bronchial epithelium as well as from each other. The individual BCH and BCH that copresented with SM were more similar to each other than to other bronchial lesions. The gene expression profile of SM that copresented with BCH was closer to the iBCH and BCH^{SM}, whereas SM that was codetected with dysplasia was similar to dysplasia (Fig. 1C).

Gene Expression Profiles of the Individual BCH and BCH that Copresented with SM

The iBCH showed overexpression of 1221 and underexpression of 1334 genes in comparison with the normal bronchial epithelium ($P < 0.05$; Figs. 1D, E). No significant functional enrichment was found for the upregulated genes. The downregulated genes were mainly related to the immune response (FDR < 0.05; Fig. 2A). The *RND1*, *VASP*, and *SPI1* genes were overexpressed, whereas the *TMEM81*, *VMO1*, and *KIAA1211* genes were underexpressed only in the iBCH compared with other bronchial lesions (FDR < 0.05; Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A228).

The BCH that copresented with SM displayed 692 upregulated and 936 downregulated genes ($P < 0.05$; Figs. 1D, E). No specifically expressed genes and no significant functional enrichment for the upregulated genes were observed in the BCH^{SM} (FDR < 0.05). The downregulated genes were associated with the organization of the extracellular matrix and the positive regulation of cell proliferation (FDR < 0.05; Fig. 2B).

Gene Expression Profiles of SM that Copresented with BCH and SM that Co-occurred with Dysplasia

In the SM^{BCH}, we found upregulation of 2342 genes and downregulation of 1437 genes ($P < 0.05$; Figs. 1D, E). The upregulated genes were mainly involved in the regulation of G-protein-coupled receptor signaling pathways (Fig. 2C). In contrast, the downregulated genes were predominantly related to the regulation of gene

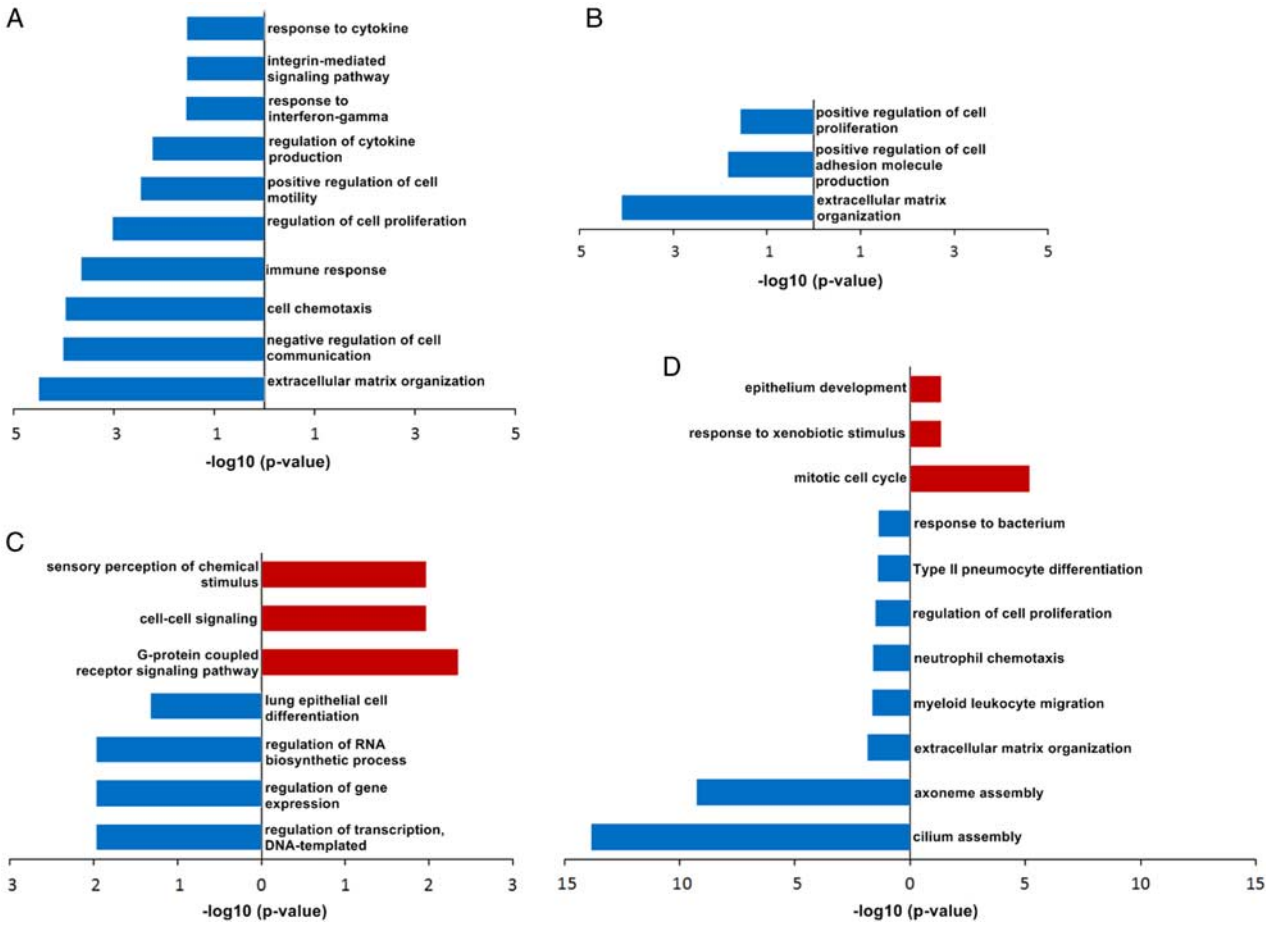

FIGURE 2. GO enrichment analysis of the genes expressed in basal cell hyperplasia (BCH) and squamous metaplasia (SM). A, Individual BCH. B, BCH combined with SM. C, SM that copresented with BCH. D, SM, copresented with dysplasia. Blue color indicates downregulated genes; red color, upregulated genes. full color online

expression and the lung epithelial cell differentiation (FDR < 0.05; Fig. 2C). The *BHLHB9*, *EXD2*, *ALG10B*, *ATPAF1*, *FOXC1*, *HOMER1*, *DIAPH2*, and *MTERFD3* genes were underexpressed only in the SM^{BCH} compared with other bronchial lesions (FDR < 0.05; Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/AIMM/A229).

The SM^{D} showed upregulation of 1909 and downregulation of 2205 genes ($P < 0.05$; Figs. 1D, E). The upregulated genes were related to the cell cycle, the response to xenobiotics, and the epithelium development (FDR < 0.05; Fig. 2D). The downregulated genes were prevalently associated with the cilium assembly and the immune cell migration and chemotaxis (FDR < 0.05; Fig. 2D). A number of specific genes were observed to be expressed in the SM^{D}. In particular, the *KHK*, *TRIM67*, *HOXD1*, *UGT2B15*, *IPO9*, and *PCDH18* genes were significantly overexpressed, whereas the *CCDC114*, *ITPR2*, *MAP7D2*, *IGLL1*, *CPQ*, *POMT1*, and *DLL1* genes were underexpressed in the SM^{D} compared with other bronchial lesions (FDR < 0.05; Supplementary Table 3, Supplemental Digital Content 3, http://links.lww.com/AIMM/A230).

Gene Expression Profile of Dysplasia

In dysplasia, we detected upregulation of 1917 and downregulation of 2250 genes ($P < 0.05$; Figs. 1D, E). The upregulated genes were enriched for cell cycle and division processes (FDR < 0.05; Fig. 3). The downregulated genes were mainly involved in the cellular chemotaxis and inflammation, in the organization of the extracellular matrix, and in the cilium organization (FDR < 0.05; Fig. 3). A significant number of genes were specifically overexpressed in dysplasia: *MS4A3*, *BTN1A1*, *KCNG3*, *SYNPO2L*, and others. In addition, dysplasia showed a large proportion of the specific underexpressed genes: *ADAM8*, *PCDH10*, *XAGE2*, *LIFR*, etc. (FDR < 0.05; Supplementary Table 4, Supplemental Digital Content 4, http://links.lww.com/AIMM/A231).

Immunohistochemical Validation of the Specific Genes Expressed in SM and Dysplasia

On the basis of the gene expression results, we selected the *CCDC114* and *MAP7D2* genes that were most significantly downregulated in SM that copresented with dysplasia in comparison with other bronchial lesions. The decision to choose the SM^{D} was based on the possibility of finding IHC markers of the oncogenic phase, that is, the progression of metaplasia to dysplasia. In addition, we focused on the *LIFR* gene that was underexpressed more than 6-fold in dysplasia compared with other bronchial changes.

In the normal bronchial epithelium, CCDC114 and MAP7D2 proteins were expressed in cilia cells, whereas the LIFR predominantly in the apical part. Overall, CCDC114, MAP7D2, and LIFR protein expression was found to be decreased from BCH to dysplasia (Fig. 4). Nevertheless, CCDC114 and MAP7D2 proteins were differentially expressed between the SM^{BCH} and SM^{D}. The most significant differences were observed in the

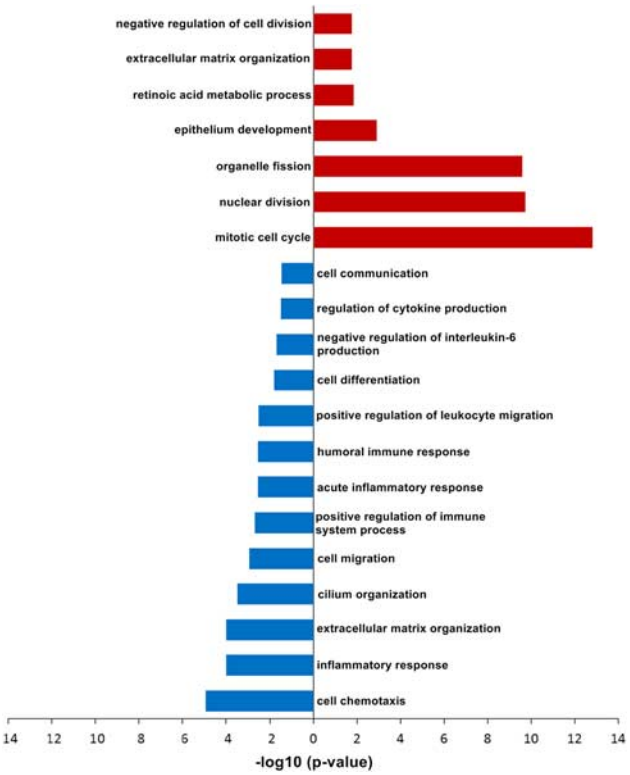

FIGURE 3. GO enrichment analysis of the genes expressed in dysplasia. Blue color indicates downregulated genes, red color, upregulated genes. full color online

CCDC114 expression that was diffuse in the SM^{BCH} and focal in the SM^{D}. The LIFR protein was found to be underexpressed not only in dysplasia, as expected, but also in the SM^{BCH} and SM^{D}. Surprisingly, the LIFR expression differed between BCH forms, demonstrating the diffuse pattern in the iBCH and the strongest reactivity in the cilia of BCH^{SM} (Fig. 4).

DISCUSSION

Squamous carcinoma of the lung develops during the premalignant changes in the bronchial epithelium: BCH, SM, and dysplasia. However, this process is not fatal; it can stop at any precancerous stage and remains so indefinitely, and then either progresses or regresses. Despite the progress in understanding the molecular biology of the precancerous process,[4,5,19] the mechanisms of its different "scenarios" remain to be elucidated.

In this work, we analyzed the gene expression profiles of BCH and SM as well as dysplasia depending on their cooccurrence in the bronchi of NSCLC patients. The study was based on the hypothesis that the combinations of the bronchial lesions reflect the different "scenarios" of the premalignant process: iBCH—the stoppage at the stage of hyperplasia, BCH plus SM—the progression of hyperplasia to metaplasia, and SM plus dysplasia—the progression of metaplasia to dysplasia (oncogenic phase). This hypothesis arises from the data that BCH^{SM} shows a

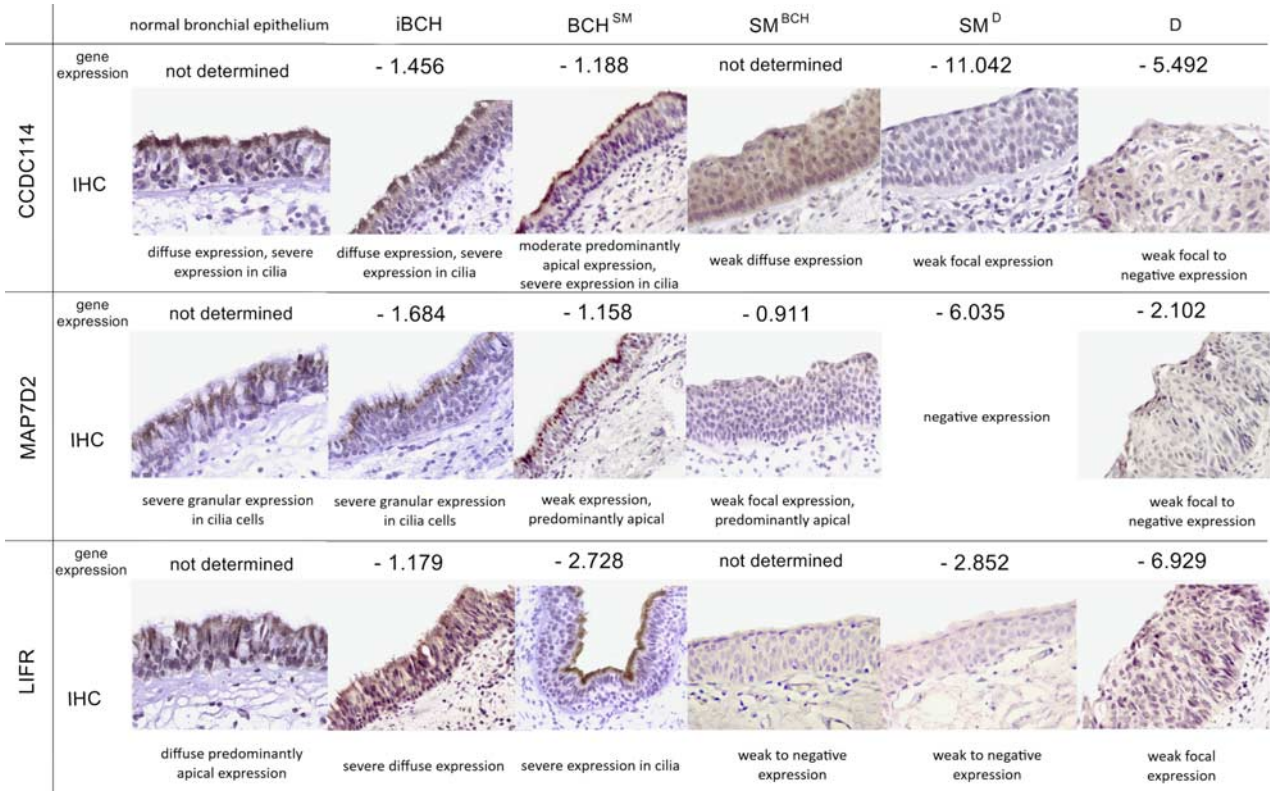

FIGURE 4. CCDC114, MAP7D2, and LIFR expression in the bronchial lesions according to the microarray data and immunohistochemical staining. BCH^{SM} indicates basal cell hyperplasia that copresented with squamous metaplasia; iBCH, individual basal cell hyperplasia; IHC, immunohistochemical staining; SM^{BCH}, squamous metaplasia that copresented with basal cell hyperplasia; SM^{D}, squamous metaplasia that copresented with dysplasia. full color online

pronounced expression of Ki-67 and p53 and a decrease in the activity of differentiation marker CD138 compared with iBCH.[12] Moreover, BCH^{SM} is associated with recurrence in NSCLC patients probably due to the similarity of inflammatory reactions occurring in the copresence of BCH and SM and provoking the development of NSCLC recurrence.[12]

Differences in gene expression were found both between the iBCH and BCH that copresented with SM and between SMs co-observed with BCH and dysplasia. The most distinctive feature of the iBCH was downregulation of the genes involved in the immune response, particularly in the regulation of cytokine production, and the reaction to them. Previously, we found that inflammatory infiltrate surrounding the iBCH shows the lowest number of $CD138^{+}$ plasma cells and the largest amount of $CD68^{+}$ macrophages compared with other bronchial lesions.[20] Probably, a low ability to support inflammation and the corresponding inflammatory milieu may inhibit the progression of BCH to SM. In contrast, BCH^{SM} showed the decrease in the extracellular matrix organization and the positive regulation of cell proliferation. However, the proliferating cells were previously found to be more in the BCH^{SM} than in the iBCH.[12] Unlike iBCH, the BCH^{SM} did not demonstrate downregulation of the genes associated with inflammation. It may indicate the development of an inflammatory microenvironment around the BCH^{SM} and its potential role in the progression of hyperplasia to metaplasia, as previously suggested.[20,21] This assumption is also supported by the considerable expression of the LIFR (LIF receptor) in the cilia of BCH^{SM}. Leukemia inhibitory factor (LIF) is a proinflammatory cytokine belonging to the IL-6 family that is capable of inhibiting cell differentiation, promoting cell proliferation, inducing the acute-phase protein synthesis, and affecting the cell recruitment into the area of damage or inflammation.[22] Most likely, LIFR expression in the ciliated cells makes the BCH^{SM} more susceptible to polyfunctional effects of the LIF cytokine. Interestingly, according to our data, the LIFR was significantly underexpressed in metaplasia and almost completely lost in dysplasia.

The main differences between SM^{BCH} and SM^{D} were shown at the level of the cilia assembly. In particular, only SM^{D} demonstrated the significant decrease in the cilia and axoneme assembly that may indicate the inability of this metaplasia to regress to BCH. This notion is supported by the more striking expression of the CCDC114 and MAP7D2 proteins in SM^{D} than in SM^{BCH}. The CCDC114 protein is a component of the outer dynein arm–docking complex (ODA-DC) in ciliated cells.[23] The knockdown of CCDC114 was recently found to significantly inhibit ciliogenesis.[24] The MAP7D2 protein is

expressed mainly in epithelial cells and is associated with the dynamics of microtubules.[25] In addition, SM^{D} was more similar to dysplasia in gene expression profile than to SM^{BCH} and, like dysplasia, showed the enrichment of cell proliferation processes. Thus, the above-mentioned results prove the hypothesis that SM^{D} has more potential to progress to dysplasia than SM^{BCH}. In addition, the loss of CCDC114 and MAP7D2 in metaplasia may serve as an indicator of its progression to dysplasia.

Taken together, this study shows the significant gene expression differences both between the individual BCH and BCH combined with SM and between SMs that co-occurred with BCH and dysplasia in the bronchi of NSCLC patients. These differences support the hypothesis that the combinations of the bronchial lesions mirror the different "scenarios" of the premalignant process as well as explore the mechanisms underlying the progression of hyperplasia and metaplasia. This hypothesis needs to be tested in future studies to elucidate molecular triggers of the oncogenic phase in the respiratory epithelium and the underlying inflammatory reactions in its microenvironment and to examine whether a genetic background in individuals determines progression or regression of the premalignant lesions.

REFERENCES

1. Dacic S. Pulmonary preneoplasia. *Arch Pathol Lab Med*. 2008;132: 1073–1078.
2. Kadara H, Wistuba II. Molecular biology of lung preneoplasia. In: Roth JA, Hong WK, Komaki RU, eds. *Lung Cancer*, 4th ed. Hoboken, NJ: John Wiley & Sons Inc; 2014:110–128.
3. Lantuejoul S, Salameire D, Salon C, et al. Pulmonary preneoplasia-sequential molecular carcinogenetic events. *Histopathology*. 2009;54: 43–54.
4. Nan Y, Du J, Ma L, et al. Early candidate biomarkers of non-small cell lung cancer are screened and identified in premalignant lung lesions. *Technol Cancer Res Treat*. 2017;16:66–74.
5. Ooi AT, Gower AC, Zhang KX, et al. Molecular profiling of premalignant lesions in lung squamous cell carcinomas identifies mechanisms involved in stepwise carcinogenesis. *Cancer Prev Res (Phila)*. 2014;7:487–495.
6. Valentine EH. Squamous metaplasia of the bronchus; a study of metaplastic changes occurring in the epithelium of the major bronchi in cancerous and noncancerous cases. *Cancer*. 1957;10:272–279.
7. Sozzi G, Pastorino U, Moiraghi L, et al. Loss of FHIT function in lung cancer and preinvasive bronchial lesions. *Cancer Res*. 1998; 58:5032–5037.
8. Van Den Broeck A, Brambilla E, Moro-Sibilot D, et al. Loss of histone H4K20 trimethylation occurs in preneoplasia and influences prognosis of non-small cell lung cancer. *Clin Cancer Res*. 2008;14: 7237–7245.
9. Bennett WP, Colby TV, Travis WD, et al. p53 protein accumulates frequently in early bronchial neoplasia. *Cancer Res*. 1993;53:4817–4822.
10. Pankova OV, Perelmuter VM, Savenkova OV. Characteristics of proliferation marker expression and apoptosis regulation depending on the character of disregenerator changes in bronchial epithelium of patients with squamous cell lung cancer. *Sib J Oncol*. 2010;5:36–41.
11. Giroux V, Rustgi AK. Metaplasia: tissue injury adaptation and a precursor to the dysplasia-cancer sequence. *Nat Rev Cancer*. 2017; 17:594–604.
12. Pankova OV, Denisov EV, Ponomaryova AA, et al. Recurrence of squamous cell lung carcinoma is associated with the co-presence of reactive lesions in tumor-adjacent bronchial epithelium. *Tumour Biol*. 2016;37:3599–3607.
13. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol*. 2011;6:244–285.
14. Travis WD, Brambilla E, Burke AP, et al. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: IARC Press; 2015.
15. Kerr MK. The classification of pre-invasive lesions. In: Cagle PT, Beasley MB, Dacic S, Kerr KM, Allen TC, Chirieac LR, Borczuk AC, eds. *Molecular Pathology of Lung Cancer*. New York, NY: Springer; 2012:35–52.
16. Zavyalova MV, Denisov EV, Tashireva LA, et al. Phenotypic drift as a cause for intratumoral morphological heterogeneity of invasive ductal breast carcinoma not otherwise specified. *Bio Res Open Access*. 2013;2:148–154.
17. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5:R80.
18. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. 2000;25:25–29.
19. Mascaux C, Laes JF, Anthoine G, et al. Evolution of microRNA expression during human bronchial squamous carcinogenesis. *Eur Respir J*. 2009;33:352–359.
20. Pankova OV, Perelmuter VM, Savenkova OV, et al. Characteristics of bronchial mucosal inflammatory response in sites of basal cell hyperplasia and squamous metaplasia combined with squamous cell lung cancer. *Sib J Oncol*. 2012;5:28–33.
21. Herfs M, Hubert P, Poirrier AL, et al. Proinflammatory cytokines induce bronchial hyperplasia and squamous metaplasia in smokers: implications for chronic obstructive pulmonary disease therapy. *Am J Respir Cell Mol Biol*. 2012;47:67–79.
22. Chodorowska G, Glowacka A, Tomczyk M. Leukemia inhibitory factor (LIF) and its biological activity. *Ann Univ Mariae Curie Sklodowska Med*. 2004;59:189–193.
23. Knowles MR, Leigh MW, Ostrowski LE, et al. Exome sequencing identifies mutations in CCDC114 as a cause of primary ciliary dyskinesia. *Am J Hum Genet*. 2013;92:99–106.
24. Li P, He Y, Cai G, et al. CCDC114 is mutated in patient with a complex phenotype combining primary ciliary dyskinesia, sensorineural deafness, and renal disease. *J Hum Genet*. 2018;64:39–48.
25. Hooikaas PJ, Martin M, Muhlethaler T, et al. MAP7 family proteins regulate kinesin-1 recruitment and activation. *J Cell Biol*. 2018. Doi: 10.1083/jcb.201808065.