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Review

Premalignant lesions of squamous cell carcinoma of the lung: The molecular make-up and factors affecting their progression

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ABSTRACT

Squamous cell carcinoma (SCC), one of the most common forms of lung cancer, shows accelerated progression and aggressive growth and usually is observed at advanced stages. SCC originates from morphological changes in the bronchial epithelium that occur during chronic inflammation: basal cell hyperplasia, squamous metaplasia, and dysplasia I-III. However, the process is not inevitable; it can be stopped at any stage, remain in the stable state indefinitely and either progress or regress. The reasons and mechanisms of different scenarios of the evolution of premalignant lesions in the respiratory epithelium are not fully understood. In this review, we summarized the literature data (including our own data) regarding genetic, epigenetic, transcriptomic and proteomic profiles of the premalignant lesions and highlighted factors (environmental causes, inflammation, and gene polymorphism) that may govern their progression or regression. In conclusion, we reviewed strategies for lung cancer prevention and proposed new models and research directions for studying premalignant lesions and developing new tools to predict the risk of their malignant transformation.

1. Introduction

Lung cancer (LC) is the leading cause of cancer morbidity and mortality worldwide; it accounts for 9% and 17% of malignant neoplasms in females and males, respectively, and for 19% of cancer deaths [1]. High rates of LC morbidity and mortality are directly related to the early diagnosis problems and late-stage disease detection. For example, the 5-year survival rate in patients with locally advanced and metastatic LC is 26–29% and 4–5%, respectively, whereas this rate amounts to 51–56% for localized lung cancer [2,3]. The situation was improved after identification of activating mutations in the epidermal growth factor (*EGFR*) gene associated with LC [4] and the development of tyrosine kinase inhibitors (TKI) [5]. However, most patients with *EGFR* activating mutations develop resistance to TKIs within 9–11 months, which is due to the emergence of new mutations [6–9]. Moreover, prescription of TKIs is reasonable only for patients with lung adenocarcinoma for which the rate of *EGFR* activating mutations reaches 20–40% [10].

Squamous cell carcinoma (SCC) of the lung is associated with smoking and accounts for 30% of all LCs [11]. The 5-year survival of patients with lung SCC does not exceed 18% [1]. Although, lung SCC rate is declining in developed countries due to the efforts to reduce smoking and the introduction of cigarette filters, in some countries such as Belarus, India, the Netherlands and the Russian Federation, the incidence is still high [1]. At present, despite advances in research [11,12], there are no guidelines on determination of molecular targets in lung SCC and prescription of targeted therapy. Thus, further research is needed both for prevention of lung SCC and development of new approaches for its early detection and identification of potential therapeutic targets. In this regard, it seems important to focus research on the precancerous (premalignant) process in the bronchial epithelium, which, actually being the key event, remains poorly studied.

Abbreviations: BCH, basal cell hyperplasia; CIS, carcinoma in situ; COPD, chronic obstructive pulmonary disease; GWAS, genome-wide association study; LC, lung cancer; LOH, loss of heterozygosity; PML, premalignant lesion; SCC, squamous cell carcinoma; SM, squamous metaplasia

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2. The precancerous process preceding lung SCC

Lung SCC originates from morphological changes in the bronchial epithelium: basal cell hyperplasia (BCH), squamous metaplasia (SM), grade I–III dysplasia, and carcinoma in situ (CIS) [13,14].

BCH is diagnosed when ciliated cells and three or more layers of basal cells are presented whereas goblet cells are absent. SM is defined by replacement of the cylindrical ciliated epithelium by the squamous epithelium. Different dysplasia grades are defined: I (mild), II (moderate), and III (severe). Mild dysplasia is characterized by minimal abnormalities, slightly enlarged cells, vertically oriented nuclei, and rare mitoses. Moderate dysplasia shows more pronounced abnormalities, moderately enlarged cells, and mitoses limited to the lower third. Severe dysplasia is characterized by cellular pleomorphism, basilar zone expanded with cellular crowding into the upper third, and mitoses confined to the lower two thirds. The morphology of BCH, SM, and grade I–III dysplasia was described in more detail in previous papers [15–19].

BCH and SM are common changes (rate of up to 75–80%) in most smokers and patients with chronic obstructive pulmonary disease (COPD) [20,21]. The frequency of SM rises as exposure to tobacco smoke increases [22,23]. Interestingly, SM is more common in males than in females [19]. SM is also found in people with inflammatory processes in the respiratory epithelium who live/work under polluted air conditions and are deficient in vitamin A [15]. SM, as well as BCH, is often found at a distance from the tumor in LC patients. In this regard, SM can occur alone and in combination with BCH or dysplasia [24–26]. Like BCH and SM, dysplasia is found in smokers (rate of up to 40%), COPD (17–80%, depending on the grade), and LC patients (up to 40%) [27–29].

BCH and SM are believed to be physiologically normal (reactive, reversible) processes occurring in response to respiratory epithelium damage caused by exposure to external factors (tobacco smoke, infection, etc.) and to inflammation. The transition from metaplasia to dysplasia is considered as an oncogenic phase [30]. Dysplasia and CIS are true premalignant lesions (PMLs) associated with a high risk of lung SCC. However, since genetic abnormalities are often observed in BCH and SM, especially in smokers, they are also considered as potential PMLs [31].

Changes in the bronchial epithelium can occur not only gradually (from the normal epithelium through BCH, SM, and dysplasia to CIS) but also discontinuously, when the precancerous process can stop at any stage, remain so indefinitely, and then progress or regress. Such discontinuous evolution of the bronchial changes was reported in different papers. Ponticiello et al. showed that mild, moderate, and severe dysplasia progressed to lung SCC in 25%, 50%, and 75% of cases, respectively, within a 4-year period [32]. Breuer et al. showed that 19 out of 45 metaplasias regressed, while 26 metaplasias remained stable or progressed to grade I and II dysplasia. Of these, only 9% of metaplasias transformed to CIS or invasive cancer. Regression, stabilization, and progression were observed in 64%, 22%, and 14% of cases, respectively, with grade I and II dysplastic changes. Only 9% of grade I and II dysplasias transformed into CIS or cancer. Malignant transformation was more often detected in cases with severe dysplasia (32%). However, 52% of grade III dysplasias regressed, and 16% of the dysplasias remained stable [33]. Similar results were obtained in other studies (discussed in detail in [34]).

The discontinuous evolution of the bronchial changes was hypothesized to be reflected in combinations of respiratory lesions that are observed in the bronchi of LC patients [24]. In particular, individual (isolated) BCH may mirror the stoppage of the precancerous process at the stage of hyperplasia, the co-presence of BCH and SM – the progression of hyperplasia to metaplasia, and the co-occurrence of SM and dysplasia – the progression of metaplasia to dysplasia. This assumption is supported by the fact that isolated and co-presented forms of BCH and SM are recurrently observed in LC patients, show the specific immunohistochemical profile, and have a high clinical significance [24,25,35]. Moreover, dysplasia combined with SM was found to progress to cancer rather than isolated dysplasia. In contrast, isolated dysplasia regresses in most cases [35].

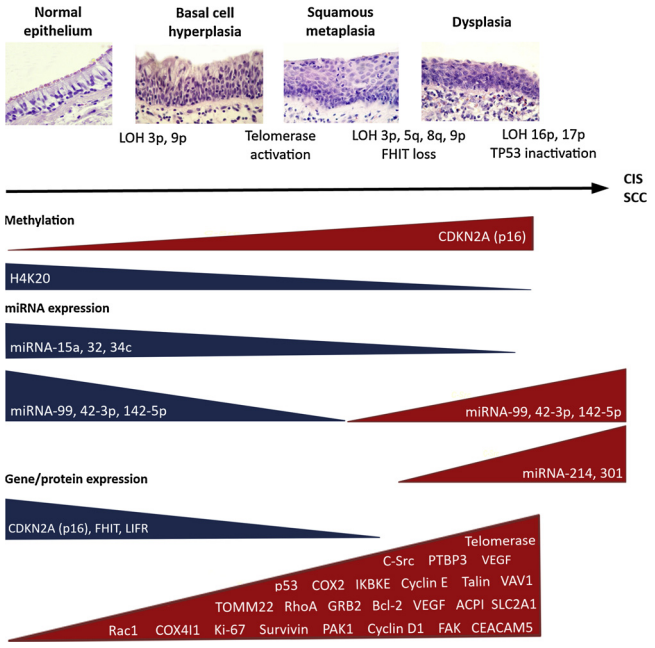

Fig. 1. Molecular alterations during of the premalignant process in the respiratory epithelium. LOH, loss of heterozygosity; CIS, carcinoma in situ; SCC, squamous cell carcinoma.

Therefore, each of the lesions preceding lung SCC can progress, remain unchanged, and partially or completely regress. Progression takes a period of several months to several decades, but the rate of progression increases as the premalignant process becomes more severe. In cases with severe dysplasia/CIS, progression is more likely. Investigation of the mechanisms underlying the precancerous process and associated with various scenarios of the evolution of bronchial PMLs is interesting and important today.

3. A molecular make-up of bronchial epithelial cells during the precancerous process

The precancerous process is characterized by gradual accumulation of genetic and epigenetic abnormalities and a significant change in the transcriptome and proteome of bronchial epithelial cells (Fig. 1).

3.1. Abnormalities in the genetic landscape

The development of respiratory changes and their progression to dysplasia are closely related to the loss of heterozygosity (LOH) on chromosomes 3, 5, 8, and 9, and telomerase inactivation (Fig. 1) [14,36,37]. The progression of BCH towards lung SCC can be triggered by trisomy of chromosome 7 that is caused by tobacco smoke and exposure to uranium [38]. Probably, the oncogenic transformation of BCH with this chromosomal anomaly may be associated with increased proliferation due to overexpression of EGFR and c-MET proteins whose genes are located on chromosome 7 [38].

The frequency of LOH on chromosomes 3p and 9p increases during the transition of severe dysplasia to CIS [39]. This is probably due to deletion of the tumor suppressor gene *FHIT* located at the 3p14.2 locus. According to Sozzi et al., the loss of *FHIT* is the most frequent alteration in LC (73%) and in bronchial PMLs (93%) [40]. The progression of dysplasia to CIS is also associated with deletions in tumor suppressor genes *RNF20* (9q31.1) and *SSBP2* (5q14.1) and amplification of the

RASGRP3 gene (2p22.3) [41]. This suggestion is based on the fact that the loss of *RNF20* and *SSBP2* leads to genomic instability and malignant transformation, respectively, while amplification of *RASGRP3* activates the RAS signaling pathway [41]. Malignant transformation of dysplasia may also be mediated by the loss of p16 expression due to deletion or hypermethylation of the *CDKN2A* gene [42–44].

The progression of CIS to invasive cancer may be associated with LOH on chromosome 3p [39]. Because LOH on 3p is much more common in CIS than in severe dysplasia, this chromosomal anomaly may be a potential marker for improving the quality of differential diagnosis of these PMLs [39]. The recent study found that the progression of CIS is strongly related to chromosomal instability. Interestingly, regressive CIS did not show genomic instability, but harbored many somatic mutations including cancer driver mutations [45].

The data regarding point mutations in BCH, SM, and dysplasia and their role in the precancerous process are extremely scarce. Two studies described *EGFR* driver mutations in the normal bronchial epithelium in LC patients [46,47]. Another study demonstrated that *KRAS* mutations occur only in dysplasia and may be associated with malignant transformation [48]. Campbell et al. found that the median number of somatic mutations in all bronchial PMLs is 0.73 per megabase (0.10–9.8 per Mb) [49]. For comparison, the mean number of mutations in lung tumors in smokers is 10.5 (4.9–17.6) [50]. CIS lesions were shown to have frequent mutations in the *TP53*, *CDKN2A*, *SOX2*, and *AKT2* genes [45].

3.2. Abnormalities in the epigenetic landscape

The methylation of genes involved in cell cycle control and tumor suppression was found to increase during the precancerous process in the bronchial epithelium (Fig. 1). *CDKN2A* (p16) methylation was observed in 13% of SM cases without dysplasia, 10% of SMs combined with grade II dysplasia, 29% of grade III dysplasias, and 25% of CIS cases. Point mutations in this gene were absent in bronchial PMLs and CIS [26]. Belinsky et al. reported more frequent methylation of the *CDKN2A* gene in CIS (50%) than in BCH (17%) and SM (24%). *CDKN2A* methylation was associated with smoking. In smokers, *CDKN2A* methylation was detected both in normal tissue samples and in SM and grade II dysplasia (18% of cases). In non-smokers, *CDKN2A* methylation was observed only at the late stages of the precancerous process [44,51]. Subsequent studies showed hypermethylation of the *RARB*, *FHIT*, *MGMT*, *RASSF1*, *DAPK1*, *APC*, and *CDH1* genes in dysplastic lesions of the bronchial epithelium [37]. CIS was found to display altered methylation and expression of several hundred genes, of which, the *NKX2-1* gene, namely its hypermethylation and underexpression, may be a driver of the progression to LC [45].

The lung premalignant process is also accompanied by changes in the histone methylation. H4K20 histone methylation (at the C3 position) was found to decrease significantly in the row: BCH – SM – dysplasia – CIS. Interestingly, changes in methylation of this histone were rarely observed in lung adenocarcinoma [52].

It is now obvious that the epigenome integrity is maintained not only by DNA methylation and histone methylation and acetylation but also by non-coding RNAs [53]. Mascaux and colleagues showed that about 69 miRNAs are differentially expressed in bronchial PMLs of non-smokers. MiRNA-32 and miRNA-34c are down-regulated from normal tissue through bronchial lesions to lung SCC. Expression of other miRNAs either changes multidirectionally or depends on the stage of the precancerous process. In general, miRNA changes during the precancerous process are opposite their dynamics during embryogenesis, particularly during lung formation. For example, miRNA-34c and miRNA-15a, which are overexpressed at the stage of lung development, are down-regulated during carcinogenesis. The levels of miRNA-99a, miRNA-142-3p, and miRNA-142-5p, which are up-regulated in embryogenesis, decreased in the early, but increased in the late bronchial PMLs. In contrast, miRNA-214 and miRNA-301 which expression is reduced during lung embryogenesis are overexpressed in severe dysplasia, CIS, and lung SCC. Thus, the precancerous process in the bronchial epithelium probably proceeds in two stages: the decrease in miRNA expression at the early (from the normal epithelium to grade I dysplasia) stage and increase in miRNA expression at the late (severe dysplasia) stage [54].

3.3. Changes in the transcriptome and proteome

Various genetic and epigenetic abnormalities undoubtedly affect the transcriptome and proteome of bronchial epithelial cells during the precancerous process (Fig. 1). Ooi and colleagues identified 626 early-stage genes initiating the precancerous process and 730 late-stage genes responsible for malignant transformation. Early-stage carcinogenesis is mainly characterized by increased ubiquitination and cell cycle activity, while the late stage is associated with high transcriptional and translational activity as well as cell migration and transformation. In addition, the carcinogenesis is accompanied by increased activity of the processes associated with cell survival and proliferation and decreased regulation of the cell death mechanisms. These changes in gene expression are probably associated with p53 inhibition and MYC activation. Interestingly, the *TP53* and *MYC* gene expression did not change significantly during carcinogenesis. This observation may indicate that the activity of these proteins changes at the post-transcriptional level [55]. Therefore, it may be supposed that transcriptome reorganization during lung carcinogenesis, particularly activity of different genes at the early and late stages of the precancerous process, may be associated with a two-step change in the miRNA profile that was described by Mascaux et al. [54] and considered above.

Significant transcriptomic and proteomic changes in bronchial PMLs have also been shown in other studies. Transcriptome analysis revealed that activity of DNA repair and HIF1A-mediated processes is increased at the precancerous stages [56]. In contrast, activity of signaling pathways regulated by STAT3, JAK/STAT, RAC1, and NCAM1 as well as processes of collagen formation and extracellular matrix organization is decreased in bronchial PMLs [56]. Proteomic profiling demonstrated enrichment of the following processes during the precancerous process: energy metabolism and oxidative stress, actin cytoskeleton remodeling (through Rho-GTPases), integrin-mediated cell adhesion, and antigen presentation to the major histocompatibility complex class I (MHC I) [57].

In general, several key genes and proteins have been described whose expression changes in bronchial PMLs. Initial studies demonstrated increased expression of the p53 [31,58], cyclin D1 [43], and Bcl-2 [31] as well as dysregulation of telomerase [59] in the row: BCH – SM – dysplasia. The anti-apoptotic protein, survivin (BIRC5), was found to be overexpressed in SM and dysplasia [60]. In contrast, the FHIT tumor suppressor was down-regulated in SM stage and was completely absent in dysplasia [40]. Dysplasia showed increased expression of the Ki-67 [61], VEGF [62,63], and cystatin A [64]. Severe dysplasia and CIS were accompanied by loss of expression of the p16 protein that inhibits the cell cycle at the G1 stage [43]. Increased telomerase activity, elevated Ki-67 proliferative index, and p53 overexpression were found to be triggers of the transformation of dysplasia into lung SCC [65].

Subsequent studies that used transcriptome and proteome profiling showed differential expression of other genes/proteins in bronchial PMLs. Ooi et al. identified three genes overexpressed during the precancerous process in the respiratory epithelim: the *CEACAM5* gene involved in cell adhesion and intracellular signaling, the *SLC2A1* gene encoding a glucose transporter (GLUT1), and the *PTBP3* (ROD1) gene that regulates pre-mRNA splicing, cell proliferation, and differentiation [55]. Nan et al. described overexpression of FAK, C-Src, integrin, GRB2, PAK1, CDC42, Rac1, RhoA, VAV1, and talin in bronchial PMLs. FAK and C-Src proteins that regulate proliferation, survival, adhesion, and migration of cells, were suggested to play a prominent role in lung SCC development [57]. Beane et al. and Li et al. demonstrated high

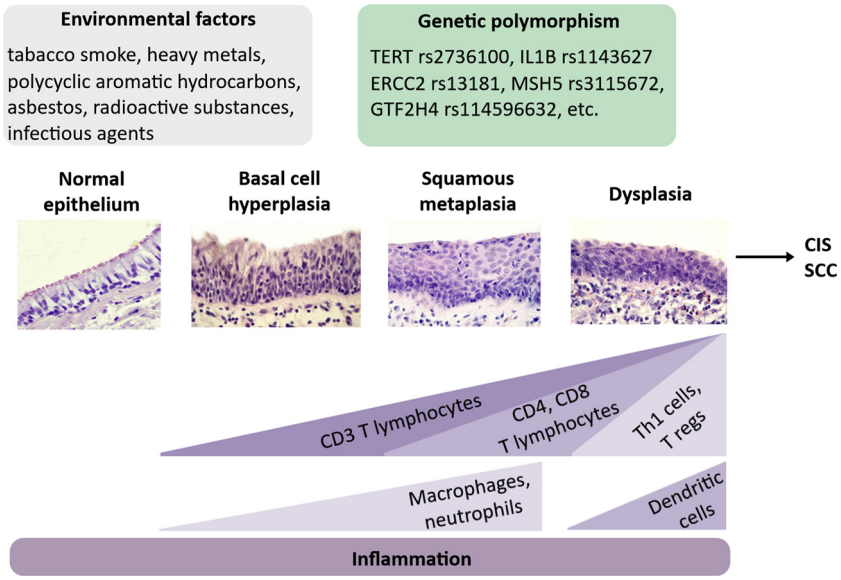

Fig. 2. Factors determining the evolution of the bronchial premalignant lesions. CIS, carcinoma in situ; SCC, squamous cell carcinoma; Th1, T helper type 1; T regs, T regulatory cells.

expression of the TOMM22 (involved in Bax-mediated apoptosis), COX4I1 (a terminal enzyme of the mitochondrial respiratory chain), and IKBKE (plays an important role in respiratory chain inflammations) in bronchial PMLs [56,66]. In our study, we showed that expression of the LIFR which is the receptor for the proinflammatory cytokine, leukemia inhibitory factor (LIF), is significantly decreased from the normal bronchial epithelium to SM and almost completely lost in dysplasia. The CCDC114 and MAP7D2 proteins were differentially expressed between SMs combined with BCH and dysplasia indicating that their loss in metaplasia may serve as an indicator of the progression to dysplasia [67].

Thus, respiratory epithelial cells show genetic and epigenetic aberrations, transcriptomic and proteomic changes already at the early stages of the precancerous process. Some of these abnormalities are associated with an increased risk of the progression and malignant transformation of PMLs. Changes in the molecular landscape of bronchial epithelial cells are probably related to the influence of exogenous and endogenous factors that are closely interrelated with each other and act as drivers of the precancerous process (Fig. 2).

4. Factors affecting the progression of bronchial PMLs

4.1. Environmental factors

PMLs in the respiratory epithelium and LC are actually caused by prolonged exposure of cells to various mutagenic and carcinogenic environmental factors. Smokers, workers in hazardous industries, and people living in a polluted atmosphere are most often affected by these factors. It is not surprising that these categories of people are at the highest risk of LC.

Currently, tobacco smoking causes 5–6 million deaths per year, 35% of which are due to cancers. Nicotine and its derivatives (carcinogenic nitrosamines) cause DNA damage by inducing oxidative stress. Nicotine also enhances cell proliferation and inhibits apoptosis by activating various signaling pathways (Ras, EGFR, Akt, XIAP, survivin, NF-kB, etc.) and induces epithelial-mesenchymal transition [68–71]. By these mechanisms, nicotine induces the development of SM and dysplasia in the respiratory epithelium and their progression to cancer [72,73]. For example, SM takes 12% of the total airway epithelial area in animals after exposure to tobacco smoke for 8 weeks [72]. Dysplasia is detected in 40% of people with a long smoking history [27].

In addition to tobacco smoke, lung carcinogenesis can be initiated by other environmental factors, such as polycyclic aromatic hydrocarbons, heavy metals, radioactive substances, asbestos, etc. At present, it is unknown how these substances affect the precancerous process in the bronchial epithelium; however, LC risk is increased in people exposed to these factors [74,75].

Infectious agents may also be considered as potential factors capable of initiating PMLs in the respiratory epithelium. Several studies demonstrate the association of *Chlamydophila pneumoniae* (*C. pneumoniae*) and *Mycobacterium tuberculosis* (*M. tuberculosis*) that are causative agents of pneumonia and tuberculosis, respectively, with LC development. *C. pneumoniae* is supposed to cause dysregulation of apoptosis, induction of superoxide radicals, and expression of TNF-α, IL-1β, and IL-8 that promote damage to lung tissue and DNA of epithelial cells [76]. It is possible that the inflammation associated with pneumonia can contribute to the development of bronchial PMLs and their malignant transformation [77,78]. The mechanism of carcinogenesis in the bronchial epithelium in the presence of *M. tuberculosis* remains not fully understood. Nalbandian et al. showed that *M. tuberculosis*-infected macrophages play a key role in tuberculosis-related LC by inducing DNA damage and by the production of an epidermal growth factor epiregulin, which is responsible for SM and tumorigenesis [79].

Thus, environmental factors associated with a high risk of LC exert a carcinogenic effect due to induction of genetic, epigenetic, and phenotypic abnormalities in respiratory epithelial cells. In addition, carcinogen-induced damage to the bronchial epithelium leads to inflammation and remodeling of the surrounding stroma. The duration of this exposure and a response of epithelial cells may determine the direction of precancerous process.

4.2. Inflammation

Chronic inflammation in response to exposure to aggressive factors, e.g., tobacco smoke or a pathogen, plays a great role in the development and progression of LC. Inflammation initiates accelerated proliferation, delayed differentiation, and metaplastic changes in the bronchial epithelium. There is evidence that these changes are mediated by pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 [80]. Inflammation is maintained by PMLs, mainly by dysplastic cells producing cyclooxygenase 2 (COX-2) that induces synthesis of pro-inflammatory prostaglandins [81]. Inflammation leads to activation of fibroblasts/

myofibroblasts and remodeling of the stroma surrounding the bronchial epithelium [82]. Thus, investigation of immune inflammatory response types during PMLs in the bronchial epithelium is a key issue to understand the biology of LC and to develop strategies for its prevention.

Several studies emphasized the important role of the innate and adaptive immune response in lung carcinogenesis. BCH and SM is accompanied by an increase in the amount of $CD3^+$ T lymphocytes, $CD68^+$ macrophages, and $CD177^+$ neutrophils [80]. In comparison with individual BCH, BCH combined with SM is characterized by a large number of $CD138^+$ plasma cells, Ki-67^+ proliferating cells, and cells expressing p53 and Bcl-2 as well as by a decrease in $CD68^+$ macrophages [83]. Such microenvironment probably supports the progression of hyperplasia to metaplasia. In contrast, a low ability to support inflammation can be a factor limiting BCH progression as proposed previously [67]. Genes associated with activation of CD8 and CD4 T cells as well as some B memory cells are progressively and linearly up-regulated from metaplasia during carcinogenesis [84]. The microenvironment of SM shows numerous proliferating cells and p53 and Bcl-2-positive cells; however, plasma cells, macrophages, and $CD117^+$ mast cells are lower compared to that in BCH and dysplasia [83]. The transition to severe dysplasia is accompanied by significant changes in expression of the genes of strong adaptive immune response via dendritic and T (Th1 and T regulatory) cells. Severe dysplasia shows pronounced immunosuppressive signals [84] and its progression to CIS is probably associated with a lack of innate and adaptive immune cells in the microenvironment [85].

The resident immune cells in the normal bronchial mucosa are characterized by some features that should be considered in studying the role of inflammation in lung carcinogenesis. A distinctive feature of the bronchial infiltrate is the presence of innate lymphocytes [86] that can sustain inflammation for a long time. However, the role of innate lymphocytes in lung precancerous process remains unclear.

Changes in the stroma of respiratory epithelium during inflammation may also provide favorable conditions for progression of PMLs. Remodeling of connective tissue and formation of structurally modified bronchial epithelium with impaired protective functions enhance inflammation and lead to the development of a hypoxic environment that initiates metaplastic changes [87]. Cancer-associated fibroblasts suppress lung dysplasia through inhibition of the activity of SOX2, restore hyperplasia, and enhance the formation of acinar-like structures [88].

Understanding the role of inflammation in lung precancerous process provides the basis to identify markers for predicting the risk of progression of PMLs to LC. Sin and colleagues found that a high level of the C-reactive protein in the blood plasma of dysplasia patients is associated with worsening of dysplastic lesions or development of new dysplastic foci [89]. Later, the same research group showed that a decrease in the surfactant protein D level in bronchoalveolar lavage is associated with progression of dysplasia in smokers [90]. Very recently, Beane et al. identified four transcriptionally distinct subtypes of endobronchial biopsies with differences in epithelial and immune processes, of which the proliferative subtype is enriched with dysplasia, shows a decrease in innate and adaptive immune response, and high risk for lung SCC [85].

4.3. Genetic polymorphism

The risk of LC, like other cancers, may depend on hereditary factors, particularly genetic polymorphism. To date, more than 1000 studies have been conducted, and more than 2900 polymorphic variants have been identified in 754 genes and chromosomal loci associated with the risk of LC [91]. According to the latest genome-wide association studies (GWAS) and meta-analyses, the risk significance was confirmed only for some of the genes and their variants: *CHRNA3* (rs6495309), *CHRNA5* (rs16969968), *BAT3* (rs3117582), *MSH5* (rs313131379), *TERT* (rs2736100), *CLPTM1L* (rs402710), etc. [91,92]. Risk variants were shown to be different between histological LC types. The largest number of high-risk genes has been found for lung SCC, many of which are located on the chromosome region 6p21 and belong to the HLA family [93].

A classic approach for identification of cancer-associated polymorphisms and genes is based on comparing the frequencies of genotypes or alleles between healthy individuals and patients. Risk variants are considered in terms of their protective or predisposing role in the development of cancer, suggesting their involvement in the malignant transformation. Meanwhile, a potential significance of these polymorphic variants in the precancerous process is involuntarily missed. For example, some allelic variants may promote progression of PMLs, while others, in contrast, prevent cells from progressing to dysplasia or CIS. In particular, it would be interesting to evaluate the influence of the *TERT* rs2736100 (A > C) polymorphism associated with a high risk of LC on expression of the corresponding protein (telomerase) in bronchial PMLs. Telomerase expression is known to be absent in normal epithelial cells, increased from metaplasia and dysplasia and reach maximum in tumor cells [94]. The rs2736100 (A > C) polymorphism leads to TERT overexpression in normal cells [95] and, thus, may predispose to LC. Interestingly, TERT expression is absent in atypical adenomatous hyperplasia that is considered as a precursor of lung adenocarcinoma [59].

As mentioned above, bronchial lesions develop in the setting of chronic inflammation that may act as both a trigger for progression and a repressor of the precancerous process. Inflammation in the lung and its type may be associated with genetic polymorphisms [96]. Interleukin-1 beta is of great interest as a pro-inflammatory cytokine that is produced by macrophages, monocytes, and lung epithelial cells and overexpressed in LC [97]. Several studies demonstrated the association between polymorphic variants of the *IL1B* gene, e.g. the rs1143627 (-31T > C) polymorphism, and the risk of LC [97–99]. The high-risk TT genotype may be related to a pronounced pro-inflammatory response and the progression of bronchial PMLs. This assumption is consistent with the data that the *IL1B* rs1143627 (-31T > C) polymorphism decreases IL1B expression [100]. Similarly, other *IL1B* gene polymorphisms may also modulate the risk of LC.

Genetic abnormalities detected in bronchial PMLs and associated with their progression may be caused by both exposure to environmental mutagenic factors and a decrease in DNA repair efficiency. Gene polymorphism is well-known to modulate DNA repair and, thus, the risk of genetic damage. To date, different polymorphic variants in DNA repair genes have been described to significantly affect the functionality of the encoded proteins. Some of them, such as *ERCC2* rs13181, *MSH5* rs3115672, and *GTF2H4* rs114596632, were found to predispose to LC [101,102]. It is possible that the progression of bronchial PMLs is more likely in carriers of these polymorphic variants due to decreased expression of the corresponding proteins, low DNA repair, and an increased risk of genetic abnormalities.

Taken together, the data discussed above indicate that the progression of bronchial PMLs is controlled by the combination of various exogenous and endogenous factors (Fig. 2). However, there are difficulties in using these factors in clinical practice for predicting the risk of precancerous process progression and lung SCC. This is probably associated both with problems of translation of the experimental findings into practice and with an insufficient predictive power of molecular markers, some of which are unacceptable due to the need for invasive procedures.

5. Approaches for therapy of patients with bronchial PMLs

There is no doubt that LC is easier to prevent than to cure. In this regard, emphasis should be shifted to suppression of the precancerous process. It is widely assumed that the progression of PMLs to cancer can be stopped by eliminating free radicals arising during exposure to tobacco smoking and long-lasting inflammation and preventing inflammation in the respiratory epithelium. However, at present, there

Table 1
Lung cancer prevention trials.

Study	Phase	N	Drug	Status	Main results
NCT03870152	II-III	111	Electrocautery ablation	Not yet recruiting	NA
NCT03300817	I	50	MUC1 vaccine	Recruiting	NA
NCT03232138	II	72	Sulforaphane	Recruiting	NA
NCT02237183	I	51	Iloprost	Recruiting	NA
NCT01717482	II	100	Metformin	Terminated (2019)	NA
NCT00780234	II	92	Pioglitazone	Completed (2017)	No differences in bronchial histology
NCT00783705	II	85	Inositol	Completed (2014)	Insignificant increase in CR and reduction in Ki-67, an increase in PD [111]
NCT00691132	II	107	Phenethyl isothiocyanate	Completed (2013)	Modest inhibition on the metabolism of lung carcinogen NNK [105]
NCT00368927	II	61	Sulindac	Completed (2010)	No sufficient benefit [112]
NCT00055978	II	112	Celecoxib	Completed (2009)	Significant decrease in Ki-67 level and reduction of lung nodules [103]
NCT00084409	II	152	Iloprost	Completed (2009)	Improvement of bronchial histology [104]
NCT00255775	NA	70	Broccoli sprout extract	Completed (2009)	No results posted
NCT00056004	II	38	Zileuton	Completed (2009)	No results posted
NCT00363805	II	178	Green tea or polyphenon E	Completed (2009)	Reduction in oxidative stress marker, 8-F2-isoprostane
NCT00321893	II	225	Budesonide	Completed (2008)	Non-significant effect on lung nodule size [113]
NCT00522197	II	90	ACAPHA	Completed (2008)	No results posted
NCT00573885	II	53	Green tea	Completed (2008)	No results posted
NCT00712647 (CARET)	IV	18314	Beta-carotene and retinyl palmitate	Completed (2005)	No chemopreventive benefit, excess LC incidence and mortality [114]
NCT00002586	II	96	13-Cis retinoic acid with or without vitamin E	Completed (2005)	Non-significant change in bronchial histology [115]
NCT00006457	I	77	Oltipraz	Completed (2004)	Terminated due to toxicity [116]
ATBC	NA	29133	Alpha-tocopherol and beta-carotene	Completed (1993)	No chemopreventive benefit, increase in LC incidence [117,118]

ACAPHA, a combination of six herbs; CR, complete response (regression of all dysplastic lesions found at baseline to lesions that were no worse than hyperplasia and no new dysplastic lesions that were mild dysplasia or worse); LC, lung cancer; NA, not applied; PD, Progressive disease (progression of one or more sites by two or more grades or new dysplastic lesions that were mild dysplasia or worse).

are no effective strategies to prevent LC. Many clinical trials have been conducted, but the results are unconvincing (Table 1). Promising results have been shown using the COX-2-selective inhibitor, celecoxib [103], pulmonary vasodilator, iloprost [104], and chemopreventive phytochemical phenethyl isothiocyanate [105]; however, further investigations have not been continued excepting iloprost that is now studied in preventing LC in former smokers (NCT02237183). Two studies are ongoing to evaluate the chemopreventive efficacy of phytochemical antioxidant, sulforaphane (NCT03232138), and MUC1 peptide-Poly-ICLC vaccine (NCT03300817). One study (NCT03870152) started this year and focuses on delaying the progression of high-grade lung lesion (s) to invasive LC by electrocautery. The results of these studies are eagerly awaited.

Checkpoint blockade immunotherapy can be used at the precancerous stage when immune-inflammatory reactions are not diverse as in cancer [106]. It was found that PD-L1 expression is induced in bronchial epithelial cells by cigarette smoking and the carcinogen benzo(a)pyrene [107] and elevated in premalignant airway cells [108–110], whereas anti-PD-L1 antibodies significantly suppress carcinogen-induced LC [107]. Thus, checkpoint blockade may be a successful approach to prevent progression of lung PMLs; however, the high cost of this therapy limits its use in the treatment of patients at high risk of LC.

Hence, further studies are needed to find novel chemopreventive agents and to develop highly effective strategies for the prevention of LC. In this regard, the focus should be on molecular alterations in bronchial PMLs and immune inflammatory response types in their microenvironment.

6. Conclusions

Many studies described different genes, proteins, miRNAs, etc. involved in the lung precancerous process; however, we do not still have effective tools to predict the risk of progression of bronchial changes to lung SCC [85]. In this regard, it seems reasonable to use new models for understanding the molecular architecture of bronchial PMLs. The key issue is to identify subjects with progressive PMLs and to prevent their transformation to invasive cancer. In our studies, we developed the hypothesis that individual and combined forms of PMLs in the bronchi of LC patients reflect the different “scenario” of the precancerous process, whereas their diagnosis represents a routine and effective method to distinguish individuals with low or high probability of the progression of PMLs [24]. In accordance with this hypothesis, we recently proposed the potential mechanisms underlying the progression of BCH to SM and SM to dysplasia [67].

In 2016, a multidisciplinary and inter-institutional project, the Pre-Cancer Genome Atlas (PCGA), was announced [119]. By now, four molecular subtypes of PMLs have been identified, one of which, proliferative, is associated with bronchial dysplasia and high risk of developing LC [85]. The molecular landscape of CIS has been elucidated and chromosomal instability was defined as a key driver of the progression to LC [45]. In fact, the PCGA has opened up a new era in precision medicine focused on early detection and prevention of cancer [119].

Declaration of Competing Interest

The authors declare no conflict of interest.

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