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Evolving Concepts in Lung Carcinogenesis

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Abstract

Lung carcinogenesis is a complex, stepwise process that involves the acquisition of genetic mutations and epigenetic changes that alter cellular processes, such as proliferation, differentiation, invasion, and metastasis. Here, we review some of the latest concepts in the pathogenesis of lung cancer and highlight the roles of inflammation, the "field of cancerization," and lung cancer stem cells in the initiation of the disease. Furthermore, we review how high throughput genomics, transcriptomics, epigenomics, and proteomics are advancing the study of lung carcinogenesis. Finally, we reflect on the potential of current in vitro and in vivo models of lung carcinogenesis to advance the field and on the areas of investigation where major breakthroughs will lead to the identification of novel chemoprevention strategies and therapies for lung cancer.

Keywords

Field of cancerization; inflammation; stem cells; genomics; epigenomics; proteomics

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THE “FIELD OF CANCERIZATION”

The “field of cancerization” refers to areas of histologically normal-appearing tissue adjacent to neoplastic lesions that display molecular abnormalities, some of which are the same as those in the tumors.[1,2] Several studies, using cytologic and molecular techniques, have established that cigarette smoking creates a field of injury in all airway epithelial cells exposed to the cigarette smoke.[2] Auerbach and colleagues first described the observation of cellular atypia throughout the airways of smokers at autopsy,[3] indicating that the cellular injury produced by smoking involves the whole respiratory tract. Recent molecular findings support the stepwise lung carcinogenesis model in which development of this field of cancerization with genetically and epigenetically altered cells plays a central role.[1,4–9] In the initial phase, injury leads to dysregulated repair by stem/progenitor cells, which form a clonal group of indefinitely self-renewing daughter cells. Additional genetic and epigenetic alterations result in proliferation of these cells and expansion of the field, gradually displacing the normal epithelium. Development of an expanding premalignant field appears to be a critical step in lung carcinogenesis that can persist even after smoking cessation.

For example, mutations in *KRAS* have been described in nonmalignant histologically normal-appearing lung tissue adjacent to lung tumors.[10] Moreover, loss of heterozygosity (LOH) events are frequent in cells obtained from bronchial brushings of normal and abnormal lungs from patients undergoing diagnostic bronchoscopy, and they have been detected in cells from both the ipsilateral tumor-containing and contralateral lungs.[11] Likewise, mutations in the epidermal growth factor receptor (*EGFR*) oncogene have been reported in normal-appearing tissue adjacent to *EGFR*-mutant lung adenocarcinoma; *EGFR* mutations occurred at a higher frequency at sites more proximal to the adenocarcinomas than at more distant regions.[8,12] More recently, global messenger RNA (mRNA) and microRNA (miRNA) expression profiles have been described in the normal-appearing bronchial epithelium of healthy smokers,[13,14] and a cancer-specific gene expression biomarker has been developed in the mainstem bronchus that can distinguish smokers with and without lung cancer.[15,16] In addition, modulation of global gene expression in the normal bronchial epithelium in healthy smokers is similar in the large and small airways, and the smoking-induced alterations are mirrored in the epithelia of the mainstem bronchus, buccal, and nasal cavities.[7,9,11,17,18]

Several studies from various laboratories have shown that large airway epithelial cells of current and former smokers with and without lung cancer display allelic loss,[17,18] *P53* mutations,[5] and changes in promoter methylation[4] and in telomerase activity of noncancerous epithelial cells.[19] By genomewide gene expression profiling of a relatively pure population of bronchial airway epithelial cells collected at the time of bronchoscopy, several physiological responses to cigarette smoke exposure have been observed, and many of these changes remain irreversibly altered even after smoking cessation.[14,16] It has also been shown that gene expression profiles in the cytologically normal bronchial airway epithelium can predict, with high sensitivity and specificity, the presence of lung cancer in current or former smokers being evaluated for clinical suspicion of lung cancer.[15] This 80-probe set combined with clinical risk factors for disease (age, smoking history, mass size, and lymphadenopathy) produces a biomarker with close to 100% negative predictive value and 95% positive predictive value.[16]

PROFILING THE FIELD OF CANCERIZATION WITH HIGH THROUGHPUT MOLECULAR ANALYSES[20]

Epigenomics

Epigenomics are high-throughput studies of epigenetic changes. Epigenetic alterations are heritable changes in gene expression without alterations in DNA sequence. These changes encompass DNA methylation, histone modifications/chromatin changes, and miRNA level alterations, and they play a vital role in the regulation of gene expression (Table 1).

DNA METHYLATION—DNA methylation at CpG dinucleotides in the 5′ region of genes is a major epigenetic mechanism of gene expression regulation.[21,22] DNA methylation is mediated by DNA methyltransferases (DNMTs). DNMT1, a maintenance DNMT, acts on preexisting hemimethylated substrates to maintain methylation patterns after DNA replication.[23] Two other DNMTs, DNMT3a and DNMT3b, act as de novo methyltransferases that catalyze the methylation of unmethylated DNA. Importantly, DNMT3a/b may also promote demethylation of DNA at promoters during cyclical demethylation and remethylation related to the transcriptional activity of these genes.

Genomic DNA *hypo*methylation, leading to genomic instability, and aberrant promoter *hyper*methylation, leading to inactivation of tumor suppressor genes,[24,25] have both been shown to be common events in human cancers. Promoter hypermethylation has been detected in the blood,[25] bronchial lavage fluid,[23] induced sputum,[26] and pleural fluid[27] of lung cancer patients. *TP16* promoter methylation was found in the sputum of smokers up to 3 years before their clinical diagnosis of squamous cell carcinoma.[28] Furthermore, methylation of the promoter region of four genes (*TP16, CDH13, RASSFIA*, and *APC*) in patients with stage I non-small-cell lung carcinoma (NSCLC) was associated with early recurrence.[29] High-throughput technology is now allowing the identification of novel target genes for aberrant methylation.[30,31] Protein expression of one of these, *OLIG1*, was found to correlate significantly with survival in lung cancer patients.[32]

HISTONE MODIFICATIONS AND CHROMATIN CHANGES—Chromatin structure is critical in the regulation of gene expression, and alterations in its structure have been linked to changes in DNA methylation, histone methylation, and acetylation patterns, depending on the target gene. The acetylated state of histones is associated with transcriptional activity, and active histone acetylation has been shown to play a role in reexpression of silenced tumor suppressor genes.[33] Recent studies indicate that histone deacetylase inhibitors have antitumor activity against NSCLC.[34–36] In addition, histone demethylases act to remove methyl groups and have been linked to several cellular processes, including DNA repair, replication, transcriptional activation, and repression.[37]

miRNAs—miRNAs are small, noncoding RNA molecules that play important roles in the epigenetic control of diverse cellular processes by altering the translation of proteins from mRNAs. miRNAs have emerged as key posttranscriptional regulators of gene expression, involved in many physiological and pathological processes, such as proliferation, differentiation, death, and stress resistance, by altering levels of gene expression.[38] A single miRNA can target many different mRNAs, and an mRNA can be targeted by multiple miRNAs, thereby creating a complex network of molecular pathways in cells. Interestingly, widespread downregulation of miRNAs is commonly observed in human cancers and has been linked mechanistically to promotion of cellular transformation and tumorigenesis. More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, frequently amplified or deleted in human cancer, resulting in frequent copy number alterations, suggesting that differences in miRNA expression may be induced by

genomic alterations. Therefore, miRNAs are also suspected to play a role as oncogenes or tumor suppressor genes.[39]

In a study analyzing NSCLC and corresponding normal lung tissues, high *hsa-mir-155* and low *hsa-let-7a-2* expression were found to correlate with poor survival in lung adenocarcinomas ($p < 0.033$). In another study, low *let-7* expression was also significantly associated with shorter survival ($p < 0.0003$), and overexpression of *let-7* in the A549 lung adenocarcinoma cell line inhibited lung cancer cell growth in vitro.[40] Subsequently, *KRAS* was shown to be a target of *let-7*,[41] and the significance of reduced *let-7* expression in lung carcinogenesis was further supported in studies that showed *let-7* suppressed tumor initiation in an autochthonous NSCLC model of *K-ras*^{G12D} transgenic mice, which was effectively rescued by ectopic expression of *K-ras*^{G12D} lacking the 3′ UTR.[42] *let-7* also inhibited in vitro and in vivo growth of *K-ras*^{G12D}-expressing murine lung cancer cells and human lung cancer xenografts.[43]

In addition to *let-7, miR-17–92* has also been implicated in the pathogenesis and progression of lung cancer because they both appear to affect the maintenance of “stemness” and cell cycle regulation. In addition to the complex regulatory networks related to miRNAs, other noncoding RNAs have been found to be important in gene regulation. For example, snoRNA has been demonstrated to have an miRNA-like function,[44] and miRNAs may have a novel RNA decoy function[45]. The multiple targets of each miRNA, in addition to the regulatory effects of many noncoding RNAs other than miRNAs, result in extremely complex regulatory networks present in normal and cancer cells. The challenge is to target these regulatory networks to reset the cells to the normal state and remove the regulatory signals associated with the cancerous state.

Genomics and Transcriptomics

Genomics refers to high throughput studies of genetic alterations. These technologies use global gene-expression profiles to develop gene signatures that attempt to determine patient prognosis independent of their clinical staging. These technologies have also been used to develop gene signatures that predict the development of lung cancer in high-risk populations and to predict their response to chemotherapy. There are now more than 35 gene signatures that have been published utilizing a mixture of four to 133 gene combinations to predict survival, recurrence, and metastasis. These signatures were recently reviewed in detail.[46] There is considerable discrepancy in the literature, where many different gene expression profiles with good predictive value for NSCLC are described, but the profiles do not necessarily overlap. This suggests that there may be many biomarkers for predicting outcome and that many of these genes may be functionally important in determining the aggressive behavior of a tumor.

Chromosome abnormalities often correlate with molecular abnormalities and provide a starting point for gene discovery and characterization in the context of a specific disorder. In cancer biology, chromosomal abnormalities carry diagnostic, prognostic, and predictive value of response to treatment. Most solid tumors are genetically unstable and may have losses or gains of whole or large portions of chromosomes, as well as DNA sequence changes of any length attributable to insertion or deletion of the microsatellite one- to four-base DNA repeating units within a tumor.[47] Measures of these genetic variations can also be used to identify novel candidate genes for lung cancer. CGH (comparative genomic hybridization) arrays, based on the high density of bacterial artificial chromosome clones, are used to study genomic copy number variations at high resolution.[48–50] Single-nucleotide polymorphism (SNP) arrays allow accurate measurement of cancer-specific LOH, polymorphisms and copy number variations in a high-throughput manner. In lung cancer, amplification of chromosome 3q is one of the most frequent changes observed, and it is also

an early event in lung carcinogenesis, as well as in aerodigestive tract tumors.[51,52] It is found in early stages of lung cancer development, including severe bronchial dysplasia, and is maintained throughout the progression of cancer.[53] In addition, novel high-throughput sequencing techniques allow for genomewide association studies (GWASs) and have been used to identify common low-penetrance alleles influencing NSCLC risk.[54] For example, two SNPs significantly associated with lung cancer risk have been identified in the chromosomal region 15q25.1, the site of *CHRNA3* and *CHRNA5* (nicotinic acetylcholine receptor α subunits 3 and 5) and *PMSA4* (proteosome α 4 subunit isoform 1), genes that encode protein subunits expressed by airway epithelial cells and are known to bind potential lung carcinogens.[55] Two other large genetic epidemiological studies reported very similar results, further suggesting that this genomic region is important in the pathogenesis of lung cancer.[56]

Previous work has demonstrated that gene expression profiles of histologically normal bronchial airway epithelial cells collected from smokers and former smokers undergoing medically indicated bronchoscopy for suspicion of lung cancer are different between patients with lung cancer and those with a benign diagnosis. The expression differences of 80-probe sets can serve as a biomarker that predicts the lung cancer status of independent samples ($n = 52$) with 83% accuracy. This biomarker is considerably more sensitive for detecting earlystage lung cancers than bronchoscopy.[15] Importantly, the accuracy of the biomarker is independent of current or cumulative tobacco-smoke exposure and other clinical risk factors for lung cancer,[16] suggesting that the biomarker measures some aspect of cancer physiology that is otherwise clinically occult. Consistent with the notion that cancer-specific patterns of gene expression in bronchial airway epithelial cells reflect a carcinogenic process, the PI3K pathway was recently shown to be activated in bronchial airway epithelial cells from patients with lung cancer at both the gene expression level and the biochemical level.[57] These data suggest that bronchial airway epithelial cells from current and former smokers with lung cancer exhibit cancer-specific properties that can be detected via gene expression profiling and that these can serve as the basis for lung cancer diagnostic biomarkers. Importantly, levels of mRNA do not always correspond with the protein levels due to posttranscriptional modulation of proteins or changes in degradation rates of proteins. It is therefore important to perform proteomic studies in parallel to complement the gene expression data.

Proteomics

Proteomics is the large-scale study of proteins, particularly of their structure and function. Several high-throughput technologies have been developed and recently reviewed.[20] Posttranslational modifications of proteins, such as phosphorylation, glycosylation, and proteolytic processing, are common events that have the potential to significantly modify protein function and to confer cellular or tissue specificity. Unlike genomic analysis, proteomic analysis has the ability to detect these modifications. In a study using a phosphoproteomic approach based on phosphopeptide immunoprecipitation and analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS), tyrosine kinases of known oncogenes (eg, *EGFR* and *c-MET*) implicated in NSCLC carcinogenesis, as well as novel kinases (eg, *PDGFRa* and *DDR1*), were identified.

Protein signals have been found that allow the classification of lung tumors by histology, the distinction of primary tumors from metastases, and the identification of nodal involvement with 75% accuracy. A 15-signal signature has also been developed that can classify patients into good and poor prognostic groups.[58] Specific protein expression patterns have also been associated with areas of normal airway histology, premalignant lesions, and invasive lung cancers with ~90% accuracy.[59]

INFLAMMATION IN THE PATHOGENESIS OF LUNG CANCER

Chronic inflammation in numerous organ sites increases the risk for cancer development to such an extent that inflammation is now considered the "seventh hallmark of cancer."[60] The link between inflammation and lung carcinogenesis is well established.[61,62] Cigarette smoke, in particular, is a potent inducer of lung inflammation and plays a key role in lung carcinogenesis.[61,62] Several changes are seen in the airways that are associated with chronic inflammation, including alterations in cytokines, chemokines and growth factors released by alveolar macrophages, lymphocytes, neutrophils, endothelial cells, and fibroblasts. Inflammation of the airway targets the epithelium for injury, which further drives an abnormal inflammatory response.

Cyclooxygenase 2

Cyclooxygenatse 2 (COX-2) is expressed constitutively at low levels in the lung. Its expression is upregulated early after injury in response to cytokines, growth factors, and other stimuli, and COX-2 is an important factor in lung carcinogenesis. Cytoplasmic COX-2 expression is upregulated in both adenocarcinomas and squamous lung carcinomas,[63] and COX-2 expression has been shown to be higher in lymph node metastases than in the primary tumors.[64,65] In addition, COX-2 expression in NSCLC has been found to be a poor prognostic indicator.[66–68]

Prostaglandin levels are increased by COX-2 during inflammation. Prostaglandins, including prostaglandin E2 (PGE2), are known to promote carcinogenesis.[63,65] Cytokines, such as interleukin-1β (IL-1β) and transforming growth factor-β (TGF-β), and growth factors, including epidermal growth factor (EGF), have been associated with induction of high expression levels of COX-2. Oncogenic events, such as mutant *KRAS* or loss of *P53*, hypoxia, and tobacco-specific carcinogens, have also been associated with elevation of COX-2.[63,69–72] Persistence of elevated levels of COX-2 in lung cancer cells is associated with loss of IL-10 receptor expression and constitutive nuclear localization of STAT (signal transducer and activator of transcription)-6.[73,74] Elevation of COX-2 and PGE2 levels have been found to promote carcinogenesis by promoting apoptosis resistance,[75] proliferation,[76] immunosuppression,[77] angiogenesis,[78] invasion,[79] and epithelial mesenchymal transition (EMT).[80]

There is a diversity of prostaglandin receptors that mediate the downstream signaling of prostaglandins. In lung cancer, the effects of COX-2 on PGE2 levels that then act via prostanoid receptors have been found to be important. The prostanoid receptors are part of the superfamily of G protein-coupled receptors, designated as EP1, EP2, EP3, and EP4. PGE2, and its signaling through the EP4 receptor, has been shown to mediate invasion in NSCLC. Inhibition of COX-2 in tumors has been shown to diminish matrix metalloproteinase (MMP)-2, CD44, and EP4 receptor expression and invasion. These findings indicate that PGE2 regulates COX-2-dependent, CD44-mediated, and MMP-2-mediated invasion in NSCLC via EP receptor signaling.[64] Additionally, EP4 receptor blockade and knockdown reduced metastasis in animal models.[81] Thus blocking the COX-2-dependent PGE2 production or activity by targeting the downstream signaling pathway of COX-2, such as the EP4 receptor, may produce more profound anticancer effects than COX-2 inhibition alone.

Epithelial Mesenchymal Transition

EMT is the developmental shift from a polarized epithelial phenotype to a highly motile mesenchymal phenotype. Although this process is essential and tightly regulated in embryogenesis and development, unregulated EMT is involved in chronic inflammation,

fibrosis, and cancer progression. EMT results in changes in epithelial proteins, such as E-cadherin, which results in enhanced migration of cells, along with changes in cell shape and adhesion.[82] In addition to the development of metastases, EMT has also been found to regulate early events in carcinogenesis.[83] EMT has been linked to the development of self-renewal properties that are usually associated with stem cells.[83]

The link between inflammation and EMT progression in the development of lung cancer and the promotion of resistance to therapy is well recognized.[80,84] Several pathways have been found to affect EMT in cancer (eg, the TGF-β pathway, PI3K/Akt, ROS (reactive oxygen species), receptor tyrosine kinase/Ras signaling, and Wnt pathways).[85–87] Other inflammatory mediators, such as IL-1β and PGE2, upregulate the zinc-finger E-box–binding transcriptional repressors of E-cadherin, including Snail, Slug, and Zeb1, resulting in EMT.[80,88] COX-2 has also been found to regulate the transcription of E-cadherin in NSCLC, and a reciprocal relationship between COX-2 and E-cadherin, as well as Zeb1 and E-cadherin in NSCLC, has been described.[80] COX-2 and PGE2 overexpression resulted in a significant reduction in E-cadherin expression via a Zeb1 and Snail transcription factor-mediated mechanism, and inhibition of COX-2 resulted in rescue of E-cadherin expression.[80]

Immunosuppression

Immunosuppression may contribute to lung carcinogenesis by allowing lung cancer cells to escape immune surveillance. Tumor cells may contribute to immunosuppression by releasing suppressive cytokines, augmenting the trafficking of suppressor cells to the tumor site, and/or promoting differentiation of effector lymphocytes to a T-regulatory cell phenotype. One major impediment to effective therapy is our inadequate understanding of how lung cancer cells escape immune surveillance and inhibit antitumor immunity. In previous studies, an immune suppressive network in NSCLC that is due to overexpression of tumor COX-2 has been defined. COX-2 metabolites have been identified as mediators of immunosuppression. PGE2 promotes the CD4 + CD25 + T regulatory phenotype and increases expression of the forkhead transcription factor FOXP3 that is known to program the development and function of T-regulatory cells.[89,90]

COX-2 and Other Signaling Pathways

Studies have demonstrated that EGFR and COX-2 have related signaling pathways that may interact to regulate cell proliferation, migration, and invasion.[91] PGE2 has been found to completely overcome the growth inhibitory activity of an EGFR tyrosine kinase inhibitor (TKI) in ~40% of NSCLC cell lines.[84] This mechanism of PGE2-induced EGFR-TKI resistance in NSCLC cells lines is mediated through EGFR-independent activation of the MAPK/Erk signaling pathway. Based on these data, there are several ongoing trials assessing COX-2 in combination with TKIs and/or chemotherapy protocols for treatment of lung cancer and for chemoprevention of NSCLC.

LUNG CANCER STEM CELLS

The cancer stem cell (CSC) model of tumor development and progression refers to the presence of a population of rare cells in a tumor that have stem cell properties; namely, they are capable of self-renewal and differentiation into their progeny. In this model, the self-renewal capacity of the CSCs is responsible for maintaining tumor growth indefinitely. Other cells comprising the bulk of the tumor are actively proliferating and differentiating and are, therefore, susceptible to current conventional cancer therapies.[92–99] Consistent with this model, CSCs are considered to be tumor-initiating cells.[92–99] Recently, it was found that CSCs may not necessarily be rare cells within a tumor. Instead, the CSC could be a rare

stem cell, a progenitor cell, or a differentiated cell that has developed the ability to self-renew.[98] These tumor-initiating cells are thought to arise from cells that have dysregulated repair, resulting in indefinite self-renewal. They are associated with relapse and recurrence of cancers and poor prognosis, presumably due to resistance to chemotherapy and radiotherapy.[93–99] The contribution of CSCs to tumor resistance fits well with the natural history of lung cancer, which is characterized by a high incidence of recurrence and metastasis, leading to the highest mortality rate of all cancers. Classical validation of a CSC tumor-initiating cell population involves reconstituting the human tumor in an immunodeficient mouse, followed by the indefinite serial xenotransplantation of the CSCs. The following putative CSC populations have been identified in lung cancer (Table 2).

Bronchoalveolar Stem Cells

The lung stem cells, termed bronchoalveolar stem cells (BASCs), were first described by Kim et al.[100] BASCs express markers of both Clara cells (CCSP [Clara cell secretory protein]) and type II pneumocytes (SP-C [surfactant protein C]), are resistant to injury with naphthalene and proliferate during epithelial repair.[100] BASCs also exhibit self-renewal and are multipotent in clonal assays. Furthermore, BASCs expand in response to oncogenic *KRAS* in culture and in precursors of lung tumors in vivo. However, the human equivalent of these cells has not yet been isolated because Sca1 + populations were used in the mouse studies. As a follow-up study, Curtis et al demonstrated that Sca1 + and Sca1–populations differed in their tumor-propagating potential depending on the genotype of the primary tumor from which they were obtained.[101]

Aldehyde Dehydrogenase and CD133 as Biomarkers for Lung Cancer Stem Cells

Aldehyde dehydrogenases (ALDHs) are a family of intracellular enzymes that are thought to play a role in cellular detoxification, differentiation, and drug resistance through the oxidation of cellular aldehydes.[102,103] Recently, the expression of ALDH proteins has been observed in numerous adult stem cell populations, including hematopoietic and neural stem cells, where they may function to preserve long-lived stem and progenitor cells.[104–106] The expression of ALDH enzymes in adult stem cells is also associated with elevated ALDH enzymatic activity and correlates with CD133 expression. Jiang et al demonstrated the ability of ALDH-expressing cells to serially propagate tumors in nude mice and determined that they were resistant to chemotherapy.[107] In addition, ALDH expression was associated with poor prognosis in patients with NSCLC.[107] Eramo et al found the CD133 (Prominin-1) surface marker expression in both small-cell and non-small-cell lung tumors.[108] High numbers of CD133 + epCAM + cells were isolated from fresh lung tumor specimens and were utilized for serial tumor xenografting via subcutaneous injections into severe-combined immunodeficient (SCID) mice. The self-renewal potential of these CD133 + cells remains to be determined, but CD133 expression was found not to be prognostic in NSCLC, although it did correlate with expression of chemotherapy resistance genes.[109] Bertolini et al showed that CD133 + cells were associated with increased resistance to chemotherapy and that CD133 +/epithelial specific antigen (ESA) cells were increased in NSCLC compared with normal lung tissue and had higher tumorigenic potential in SCID mice.[110] Li and colleagues showed that dual expression of CD133 and ABCG2 was an independent predictor of postoperative recurrence for patients with stage I NSCLC and that these tumors had increased angiogenesis.[111]

Keratin 14 as a Novel Biomarker for Lung Cancer Stem Cells

Keratin 5 (K5)-expressing basal cells are considered to be progenitor cells in the adult large airways at steady state and during airway epithelial repair.[112–115] All keratin 14 (K14)-expressing cells also express K5. Although K14 + progenitor epithelial cells in the airway are important for repair, they are rarely found in the airway epithelium under homeostatic

conditions; in contrast, K5 + cells are relatively abundant.[113,114] K14 expression was found in the repairing airway epithelium, but also in premalignant lesions and a subset of NSCLC tumors.[116] The presence of K14 + progenitor cells in NSCLC tumors after chronic smoking injury was associated with increased mortality from lung cancer.[116] This is consistent with the development of dysregulated repair after injury, leading to a self-renewing K14 + progenitor cell population in premalignant lesions that could survive long enough to accumulate the genetic and epigenetic changes considered necessary for tumor development.[96] This implicates a novel putative tumor-initiating cell population in a subset of smoking-related NSCLCs with a poor prognosis.

Snail as a Novel Biomarker for Cancer Stem Cells

Upregulation of Snail and induction of EMT may represent novel signaling events driving lung carcinogenesis. While Snail, Slug, Zeb, and Twist are known to contribute to the progression of established tumors, they are increasingly recognized for their role in neoplastic transformation, as recently reviewed by Sánchez-García.[117] Mani and colleagues were the first to report that induction of EMT in immortalized human mammary epithelial cells leads to acquisition of mesenchymal traits and expression of stem cell markers.[118] More recently, LBX1, which directs expression of Snail and Zeb1, was noted to morphologically transform mammary epithelial cells and to expand the CD44 + CD24− cancer stem cell subpopulation.[119] In a study of pancreatic and colon cancers, Zeb promoted tumorigenicity by repressing stemness-inhibiting miRNAs.[120] The role of EMT in acquisition of stem cells characteristics and malignant conversion of the otherwise normal bronchial epithelium is currently being investigated.

In a recent study, squamous cell carcinoma and adenocarcinoma subtypes of NSCLC both overexpressed Snail compared with normal lung tissues.[121] Likewise, premalignant NSCLC lesions overexpressed Snail, often in association with widespread inflammation, as did the proximal and distal airways of chronic obstructive pulmonary disease–involved lungs and premalignant lesions contained therein.[122] These findings suggest the transcription factor is implicated in the earliest pulmonary carcinogenic events.

Expression of Stem Cell Signaling Pathway Genes as Biomarkers for the Presence of Lung Cancer Stem Cells

The ability of CSCs to self-renew has been attributed to the retention or reactivation of stem cell signaling pathways, such as the Notch, Wnt, and Hedgehog pathways.[123] By capitalizing on the differential expression of self-renewal signaling pathways in lung CSCs, new therapies may be employed to selectively inhibit the self-renewing cancer cell population.[124] For example, the suppression of Notch signaling in breast and brain CSCs resulted in the reduction of self-renewing stemlike tumor cell populations.[125–127] In some lung cancers, the reduction of Notch signaling by gamma-secretase inhibition has been shown to reduce tumorigenicity and colony formation in vitro; however, the effect on the lung CSC population has not been determined.[128]

CONCLUSIONS AND FUTURE PERSPECTIVES

Many important discoveries related to lung carcinogenesis have been made, but the disease is extremely complex and there are many aspects of the biology that are not well understood. Consequently, the mortality from lung cancer remains higher than that of any other cancer. This review highlights the evolving concept that inflammation in the lungs sets up a field of injury that promotes the development of lung cancer and that the entire epithelium, not just the cancerous region, is involved in the stepwise progression to lung cancer. If this is the case, then the injured airway epithelium provides an intriguing site for further investigation

and could be targeted via chemoprevention strategies. The revolution in "–omics" approaches will make highthroughput studies of this region feasible and hold the key to determining early events in carcinogenesis. Another novel concept is the idea that reparative cells in the field of cancerization represent tumor-initiating cells, which develop additive and sometimes synergistic molecular changes that result in stepwise progression to lung cancer. The exact populations of tumor-initiating cells and their aberrant signaling pathways remain to be elucidated, as do the specific genetic and epigenetic alterations in these cells that provide the irreversible event for the development of a tumor. Whether these genetic and epigenetic changes in the tumor-initiating cells will be conserved among all individuals or are variable across the population also remains to be determined and will be part of the development of personalized medicine for lung cancer.

Future discoveries in the field of lung carcinogenesis will rely heavily on modeling of the stepwise progression of disease. Currently, the two most important models of the disease are transgenic mouse models and immortalized human bronchial epithelial cell (HBEC) models. In transgenic mice, the somatic activation of *KRAS* has been shown to induce lung adenocarcinomas.[129] Likewise, somatic activation of point mutations of *P53* induced tumors, though *P53* did not, suggesting that point mutant *P53* alleles have enhanced oncogenic potential beyond the simple loss of *P53* function.[130] Most importantly perhaps, inactivation of both *KRAS* and *P53* resulted in the development of a mouse model of SCLC,[131] which will be extremely valuable for the field.

HBECs are immortalized with *CDK4* and *hTERT* and can be cloned and genetically manipulated, but they do not form colonies in soft agar or tumors in nude mice. HBECs are capable of differentiation into a pseudostratified epithelial layer, similar to that of normal human bronchial epithelium, when grown in an air–liquid interface culture model.[132,133] This is a useful model system for analyzing the stepwise progression of lung cancer. For example, HBECs manipulated to have mutant *KRASV12, P53* knockdown, or mutant *EGFR*, alone or in various combinations, acquire the ability to grow in soft agar and to invade in three-dimensional organotypic cultures.[132]

In summary, we have learned a great deal about the genetic and epigenetic changes that occur after airway injury and are found in lung tumors and the surrounding airway epithelium. Novel technologies will allow us to greatly expand our understanding of the stepwise changes that result in lung cancer and will enable us to identify which cells and molecular changes are responsible for the progression. This is likely to yield important advances for the field where the ultimate goal is development of novel therapies and chemoprevention strategies.

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Table 1

Examples of High-Throughput Techniques for Molecular Analyses

Analyte	High-Throughput Methods
Genomics	Whole genome sequencing, CGH arrays, SNP arrays
Epigenomics	miRNA microarrays/sequencing, DNA methylation arrays/sequencing (MeDIP, or bisulfite conversion)
Transcriptomics	RNA-sequencing, gene expression microarrays
Proteomics	Two-dimensional gel electrophoresis, MALDI-TOF MS, tandem MS, protein arrays, tissue microarrays

CGH, comparative genomic hybridization; SNP, single-nucleotide polymorphism; miRNA, microRNA; MeDIP, methylation dependent immunoprecipitation; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Table 2

Published Putative Cancer Stem Cells in Lung Tumorigenesis

Putative Stem Cell (Species)	Location/s in the Lung	Serial Xenografting Performed	Association with Prognosis when Present in Tumors	References
Bronchoalveolar stem cell (BASC) IF: CC10+SPC+FACS:Sca1 +CD45-Pecam- (mouse)	Bronchoalveolar duct junction	Yes	Not known	Kim et al (2005)[100]
Reparative cell IF: K14+K5 +FACS: N/A (human)	Submucosal gland duct, submucosal glands, repairing airway epithelium, preneoplastic lesions	No	p=0.003	Ooi et al (2010)[116]
CD133+IF and FACS (human)	Not known	Yes	−CD133+Not significant for prognosis	Eramo et al (2008)[108]
			−CD133+ABCG2+predicts recurrence in stage I NSCLC p=0.015	Bertolini et al (2009)[110]
			−Associated with resistance to chemotherapy	Salnikov et al (2010)[109] Li et al (2010)[111]
ALDH$^+$ (human)	Not known	Yes	p=0.009	Jiang et al (2009)[107]
			−Associated with resistance to chemotherapy	

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ALDH, aldehyde dehydrogenase; K14, keratin 14; K5, keratin 5; CC10, Clara cell secretory protein; SPC, surfactant protein C.