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Preneoplasia of lung cancer

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Abstract

As with other epithelial cancers, lung cancer develops over a period of several years or decades via a series of progressive morphological changes accompanied by molecular alterations that commence in histologically normal epithelium. However the development of lung cancer presents certain unique features that complicates this evaluation. Anatomically the respiratory tree may be divided into central and peripheral compartments having different gross and histological anatomies as well as different functions. In addition, there are three major forms of lung cancer and many minor forms. Many of these forms arise predominantly in either the central or peripheral compartments. Squamous cell and small cell carcinomas predominantly arise in the central compartment, while adenocarcinomas predominantly arise peripherally. Large cell carcinomas are not a single entity but consist of poorly differentiated forms of the other types and, possibly, some truly undifferentiated “stem cell like” tumors. The multistage origin of squamous cell carcinomas, because of their central location, can be followed more closely than the peripherally arising adenocarcinomas. Squamous cell carcinomas arise after a series of reactive, metaplastic, premalignant and preinvasive changes. However, long term observations indicate that not all tumors follow a defined histologic course, and the clinical course, especially of early lesions, is difficult to predict. Peripheral adenocarcinomas are believed to arise from precursor lesions known as atypical adenomatous hyperplasias and may have extensive in situ growth before becoming invasive. Small cell carcinomas are believed to arise from severely molecularly damaged epithelium without going through recognizable preneoplastic changes. The molecular changes that occur prior to the onset on invasive cancers are extensive. As documented in this chapter, they encompass all of the six classic Hallmarks of Cancer other than invasion and metastasis, which by definition occur beyond preneoplasia. A study of preinvasive lung cancer has yielded much valuable biologic information that impacts on clinical management.

Keywords

Lung cancer; squamous cell carcinoma; adenocarcinomas; small cell lung carcinoma; preneoplasia; carcinoma in situ; atypical adenomatous hyperplasia; tumor suppressor genes; oncogenes; apoptosis; telomerase; angiogenesis

1. Introduction

Lung cancers represent the commonest cause of cancer deaths in the world, accounting for about 1.2 million deaths per year [1]. The disease is largely preventable as most cases are due to tobacco smoking [1]. There is a close relationship between the amount of exposure to tobacco carcinogens, and the subsequent appearance of lung cancers several years or decades later [2]. During the long latent period, as with other epithelial cancers, sequential preneoplastic changes develop, and form the basis of the multistage pathogenesis of lung cancers. Such preneoplastic and preinvasive lesions are indicators of exposure and also offer opportunities for risk assessment and chemoprevention [3]. The molecular changes present in lung (and other) cancers are often complex and numerous, involving genetic, epigenetic and cytogenetic abnormalities [4], and their roles in lung cancer pathogenesis form the basis of this chapter. Although not recognized as a Cancer Hallmark, microRNAs (short RNAs regulating mRNA function) are known to play important roles in lung development and cancer pathogenesis [5], and indirectly affect several of the Hallmarks [6]. While relatively little is known about microRNAs in lung cancer pathogenesis, Dicer, a key effector protein for small interfering RNA and microRNA function, is up regulated during lung adenocarcinoma development [7].

It is important to differentiate between driver mutations (important or essential for the appearance or maintenance of the malignant state) and passenger mutations of little or no importance for the malignant phenotype [4,8]. Driver mutations may appear during the course of multistage pathogenesis and are usually maintained during tumor progression and metastasis, while passenger mutations, resulting from genomic instability, may appear throughout pathogenesis, invasion and spread. Thus our discussion is focused on driver mutations. In this chapter we describe the multistage pathogenesis of lung cancers and their histological and molecular features.

2. The development of the lung

The lung and its tumors represent a complex topic as the organ may be divided into central and peripheral compartments, and distinct tumor types arise from each compartment. An understanding of lung development is crucial for understanding this concept.

The lungs develop as an anterior outgrowth from the embryonic foregut, as does the thyroid. The location of both buds is marked by expression of the master transcription factor thyroid transcription factor or TTF1 (also called TTF1) [9]. While TTF1 is essential for lung development, in adult life it functions as the major transcription factor for the peripheral airway epithelial cells. As the lung develops, it forms a series of closed buds, lined by a single form of epithelial cell, a tall columnar glycogen rich cell with apical located nuclei. As no other cells enter the developing epithelium, we can presume that these cells represent the primordial stem cell of the lung, and that all epithelial including neuroendocrine cells are derived from this primordial stem cell. The branching developing lung becomes enveloped in mesenchyme, and epithelial-mesenchymal interaction is essential for further development and maturation. Full maturation does not occur until shortly before birth.

3. The variety of lung cancers

The World Health Organization (WHO) classification of lung cancers lists four major types, small cell lung cancer (SCLC) (about 20% of lung cancers in the USA) and the three major types of non small cell lung cancer (NSCLC), namely squamous cell, large cell and adenocarcinoma [10]. While squamous cell carcinoma is the commonest form of cancer, especially in smokers, adenocarcinoma has become the commonest form in many parts of the world, and its incidence is rising [11,12]. Several subtypes of adenocarcinoma have been

described, although the vast majority of adenocarcinomas contain elements of multiple subtypes. The subtype that has generated the greatest interest is the bronchiolo-alveolar subtype (BAC), defined as growth of neoplastic cells along pre-existing alveolar structures without evidence of stromal, vascular or pleural invasion. Thus, as strictly defined, it is a non-invasive form of cancer, and if diagnosed correctly, small resected BAC tumors have an excellent prognosis. However the widespread application of the term BAC to invasive tumors having a prominent BAC component by pathologists and clinicians has led to great confusion and misunderstanding. At the World Lung Cancer Congress in 2009, it was proposed that the term BAC be replaced with the term pulmonary adenocarcinoma in situ.

4. The concept of central and peripheral lung compartments

The lung is one of a few organs (along with the esophagus) in which distinct anatomic and functional compartments have been recognized, and these compartments give rise to specific tumor types [13]. The central compartment consists of the large and medium sized bronchi, involved in air conduction. The peripheral compartment consists of smaller airway bronchioles and alveoli. The histological structures of these compartments are very different. The central airways are lined by pseudostratified epithelium having basally located basal cells and neuroendocrine cells, and luminal tall ciliated cells and mucous secreting cells. In the terminal (respiratory) bronchioles are located short stubby ciliated cells and secretory Clara cells. The alveoli are lined by flattened type I alveolar pneumocytes (through which gas exchange occurs) and type II pneumocytes which secrete surfactant. The only cells capable of sustained division are the Clara cells and the type II alveolar cells. Both of these cell types are believed to be derived from a common bronchiolo-alveolar stem cell [14]. Thus the primordial lung stem cell is believed to give rise to the secondary stem cells of the central and peripheral compartments.

Most squamous and small cell carcinomas (SCLC) arise from the central airways, while many if not most adenocarcinomas arise from the peripheral airways. The peripheral adenocarcinomas often have a prominent bronchiolo-alveolar component, although true bronchiolo-alveolar carcinomas (BAC), as defined by the WHO classification, are non invasive carcinomas. Some adenocarcinomas arise centrally from the surface epithelium or occasionally from the bronchial glands.

5. Stem cells in lung development and cancer

The central and peripheral airways have very different structures and functions and give rise to different cancer types. Thus, although the epithelium of the lung develops from a single primordial stem cell, the central and peripheral compartments have their own specialized stem cells. In the central airways, the basal cells serve this function, and in the peripheral airways specialized Clara cells and type 2 pneumocytes function as stem cells [15]. These findings have important correlates with the origin of tumor cells. While the lung cancer stem cell remains elusive, it is reasonable to postulate that squamous cell cancers arise from transformed central airway stem cells and that adenocarcinomas arise from similar stem cells in the peripheral airways. SCLC, a neuroendocrine tumor is now believed to arise from the central airway stem cells, although the neuroendocrine cells in this location may have a different stem cell. However, the fact that all of the bronchial epithelium arises from a common primordial stem cell results in tumors having mixed squamous and adenocarcinoma (frequent) and NSCLC-SCLC tumors (relatively rare) [10].

The discovery of rare tumor cells with stem cell features first in leukemia and later in solid tumors has emerged as an important area in cancer research. It has been determined that these stem-like tumor cells, termed cancer stem cells, are the primary cellular component within a tumor that drives disease progression and metastasis. In addition to their stem-like

ability to self-renew and differentiate, cancer stem cells are also enriched in cells resistant to conventional radiation therapy and to chemotherapy [15]. The cancer stem cell model for tumor progression indicates that only a subpopulation of cancer cells possess the ability to proliferate indefinitely. While we have tantalizing clues from a large body of work from many laboratories, currently little is known about the biology of lung cancer stem cells: including their distinguishing properties and proportions in different types of lung cancer, how they evade and resist therapy, and importantly, what drives their self-renewal. In addition, it is not fully understood how clues from the tumor microenvironment might support or determine the cancer stem cell phenotype in lung cancer.

6. Premalignant and preinvasive changes in the central and peripheral airways

As with most epithelial tumors, lung cancers are preceded by a series of premalignant changes [16]. Because of the compartmentalization of the lung and the multiple histological tumors, these differ for individual tumor types and compartment location. In the large bronchi (which can be serially sampled via endoscopy), a series of squamous oriented changes leading from hyperplasia (either basal cell or mucous), squamous metaplasia, different degrees of dysplasia to carcinoma in situ and invasive cancer have been described [17]. Another change described in the bronchial mucosa is angiogenic squamous dysplasia, which results in vascular thickenings or protrusions of the bronchial mucosa, usually accompanied by overlying squamous dysplastic changes [18]. They are regarded as evidence that neoangiogenesis of the bronchial mucosa is an early premalignant change (Fig. 1).

The natural history of bronchial preneoplastic lesions is based on scant data. While conventional belief is that lesions progress along the sequential path described above, actual observations indicate that the natural history and progression of lesions is highly variable, and that the sequential pathway is not always followed [19]. Sequential biopsies of specific sites may demonstrate progression to CIS or cancer without all the intermediate stages (parallel theory of progression). Dysplastic lesions demonstrate high (>50%) spontaneous rates of regression (although some may be removed *in toto* by the biopsy procedure or by the resultant inflammatory and fibrotic reaction).

Many of the premalignant lesions cannot be detected by white light bronchoscopy, and fluorescence bronchoscopy is much more efficient at detecting such lesions [20]. A lesson learned from fluorescence bronchoscopy is the small size of many of these lesions (one to three mm in longest length). The small size of the premalignant lesions complicates studies of the natural history of such lesions, as bronchial biopsy may remove the lesions either directly or by the subsequent inflammatory and fibrotic reaction.

The development of peripheral adenocarcinomas is less well defined as sequential biopsies cannot be obtained. Our current belief is that focal lesions of type 2 pneumocytes (atypical adenomatous hyperplasias, AAH), precede the onset of non-mucinous bronchiole-alveolar carcinomas (BAC). AAH lesions are defined by the WHO as a localized proliferation of mild to moderately atypical cells lining involved alveoli and sometimes respiratory bronchioles, resulting in focal peripheral lung lesions usually less than 5 mm in size. BACs (by strict definition, are non-invasive lesions) eventually lead to invasive carcinoma (often starting in a central scar focus). Molecular studies support the concept of AAH being a premalignant lesion and BAC being a preinvasive lesion. However, because BAC can potentially occupy much of the lung and spread bilaterally via aerogenous dissemination, it is preinvasive cancer that can kill the host. Small BACs have an excellent prognosis, but are relatively rare (~5% of lung cancers). Unfortunately the term has been widely misused and applied to mixed (invasive) adenocarcinomas having a prominent BAC component. Thus the

pathology panel of the International Association for the Study of Lung Cancer at the 2009 World Congress of Lung Cancer proposed that the term be abandoned in favor of adenocarcinoma in situ (Fig. 2).

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a diffuse collection of single or small nodules of neuroendocrine cells in the respiratory mucosa. Localized extraluminal proliferations are known as tumorlets [21]. While it is tempting to postulate that these rare lesions are the precursors for SCLC, they are more likely to be precursors of carcinoid tumors. No precursor lesions have been identified with certainty for the development of small cell carcinoma of the lung (SCLC). However, microdissection studies indicate that extensive genetic damage in the accompanying normal and hyperplastic bronchial epithelium is characteristic of SCLC tumors and suggest major differences in the pathogenesis of the three major lung cancer types [22].

7. Field effects in the central and peripheral compartments of the lung

Because of the relative ease of sampling of the large airways via non-invasive or minimally invasive procedures (sputum collection, bronchioloalveolar lavage fluid, or bronchial brushes and biopsies) we know much more about the central airway preneoplastic and preinvasive lesions and their natural history. By contrast, the peripheral airways can only be sampled via invasive procedures (such as needle or open biopsies) or as incidental findings in lung cancer resection studies. However the advent of mass computerized tomography (CT) screening techniques has resulted in the identification of many known or suspected AAH lesions. While these lesions may be followed by sequential scans, their actual histological appearances (and molecular changes) may not be known.

A wide variety of molecular lesions, often numerous, are present in the carcinogen damaged lungs of smokers. These lesions are very small, and molecular changes precede the onset of pathologically recognizable changes [23,24]. The number of lesions, their size and the complexity and the number of their molecular changes increase with increasing histological severity of the lesions [23,25]. The order of appearance of such changes is not random but follows certain general guidelines [26].

In smoke damaged lungs there are numerous foci of molecularly damaged and histologically abnormal foci. They tend to be larger and more advanced in the vicinity of the tumors. Thus smoke associated lung cancers demonstrate large field effects. In the lungs of never smokers with lung cancers, the field of damage is usually much less limited, to the region surrounding the tumor. Field effects in cancers also result in multiple synchronous and metachronous tumors. These tumors present a dilemma – are they multiple primary tumors arising as a result of field cancerization or are they intra-pulmonary metastases? Molecular studies indicate that they arise by both mechanisms, but that the origin has little effect on prognosis, as aggressive surgical resection of these lesions results in a surprisingly good prognosis [27].

8. The hallmarks of cancer and their acquisition during multistage pathogenesis

In a landmark article, Hanahan and Weinberg defined the Hallmarks of Cancer [28]. They identified six acquired hallmarks that characterize most tumors. Acquisition of these hallmarks results in cancer cells losing the regulatory circuits that govern normal cell proliferation and homeostasis. Expression of the hallmark characteristics results in genome instability. In this section we examine their relationship to the multistage pathogenesis of

lung cancers. We illustrate each hallmark with one or more examples of tumor characteristics associated with deregulated growth,

8.1. Self sufficiency in growth signals

Normal cells proliferate only in the presence of signals that activate complex signaling pathways via binding to transmembrane receptors. Cancer cells bypass normal cell cycle controls by over expression or other abnormalities of components of the major signaling pathways. The complexity of these pathways and crosstalk between pathways often result in multiple downstream effects. In lung cancers, perhaps the best studied pathway is signaling via activation the epidermal growth factor receptor (EGFR) family. This pathway is deregulated, often at multiple levels, in almost all solid tumors (with the possible exception of SCLC). Activation of EGFR (the prototype of a family of four tyrosine kinase receptors) results in activation of all of the hallmarks of cancer other than limitless replication [16]. Mutations and/or amplifications of EGFR (and, occasionally) of other family members are frequent in NSCLC tumors and cell lines, and target Asiatic ethnicity, adenocarcinoma histology, never smoking status and female gender [29,30]. In contrast to cancers in never smokers, smoke exposure related adenocarcinomas seldom mutate the EGFR gene, but more frequently mutate the KRAS gene, which lies downstream of the receptor tyrosine kinase. Because either EGFR or KRAS mutations are sufficient for tumorigenesis, they are mutually exclusive [29,30].

Because driver mutations of EGFR and KRAS target adenocarcinomas, they are frequently present in AAH lesions (see above). While EGFR mutations can be detected in a limited field within tumors or in their vicinity, much less is known about the field effect of KRAS mutations. While mutations of EGFR are an early event during multistage pathogenesis, amplifications are a relatively late event, appearing at the tumor stage, and associated with tumor progression and metastasis [31–33].

Because EGFR and KRAS mutations are seldom present in squamous cell carcinomas (and never in SCLC) they have seldom been described in the premalignant or preinvasive lesions of the large airways. However, the EGFR pathway may be deregulated by mechanisms other than mutation or amplification. One of these mechanisms include release of ligands for EGFR family members, thus forming an autocrine loop, and is found in all histologic types of NSCLC including squamous cell carcinomas [33,34].

8.2. Insensitivity to antigrowth signals

In normal cells, proliferation is tightly controlled by multiple antigrowth signals. Such signals may temporarily (by forcing a quiescent state) or permanently (by inducing differentiation) remove cells from cycling. In order to divide, premalignant cells must evade these antiproliferative signals. In particular, the ability to avoid cell cycle checkpoints is paramount. Disruption of the Rb signaling circuit and inactivation of CDKN2A (p16^{INK4}) gene are frequent in many cancers. While inactivating mutations of RB are frequent in SCLC, the pathway is usually disrupted in NSCLC by inactivation of CDKN2A (p16), often by methylation but occasionally by inactivating mutations or homozygous deletions. Inactivation of CDKN2A has been described in preneoplastic lesions of central (dysplasia) [35] and peripheral (AAH) tumors [36–38]. Rb protein expression is lost in 80% of SCLC but only in 15% of NSCLC and never in preinvasive lesions. In contrast, Rb inactivation through deregulation of its phosphorylation is common in NSCLC. Two mechanisms are responsible for this deregulation: the loss of the CDK inhibitor p16^{INK4}, which negatively controls the CDK-cyclin activity, and the overexpression of cyclin D1. Inactivation of p16^{INK4} in NSCLC is mainly caused by exon 1 or 2 mutations (10%), homozygous deletions (30 to 40%) or promoter methylation (30 to 40%) [39]. There is a strict inverse relation

between Rb and P16 expression. Hypermethylation of p16^{INK4} can be detected in bronchial epithelium from smokers with a high risk, suggesting that the inactivation of p16^{INK4} occurs early in lung tumorigenesis. P16 methylation can be also detected with a very high sensitivity (one allele methylated detected in over 10⁴ normal unmethylated alleles) in exfoliated cells, and thus represents a useful tool for early detection of lung cancer [40,41].

Cyclin D1 and/or cyclin E over expression is responsible for deregulation of Rb phosphorylation in about 50% of NSCLC and is an early event in preinvasive process as it can be detected by immunostaining in half of dysplasia, increasing in frequency with their grade [38] and in nearly 70% of AAH [42] (Fig. 3). The RB gene is mutated in most SCLC cancers [43,44], although as previously discussed, the preneoplastic process in this cancer type is poorly understood.

Antigrowth signals are mainly the products of genes that function as tumor suppressors (TSGs). A common method of inactivation of suppressor genes is via methylation of one allele and deletion of the other allele. Methylation is one form of epigenetic change (i.e. one that does not result in a change in the basic DNA structure [45]). It plays a major role in many cancers with numerous genes (about 400) inactivated in the individual tumor. Each tumor type has its own signature of frequently methylated genes, although very few methylated genes are tumor specific. Methylation commences early during multistage pathogenesis, and can be detected in the sputum, bronchioloalveolar lavage fluid and in AAH lesions [46–48].

8.3. Evasion of apoptosis

Tumor growth represents an imbalance between proliferative signals and cell attrition (predominantly by apoptosis). Activation of many oncogenes either induces senescence [49] or triggers the apoptotic mechanism, and evasion of these processes may be the only mechanism by which premalignant cells (containing driver mutations) can survive. These mechanisms may not be fully activated until the malignant state, thus explaining spontaneous disappearance of some premalignant lesions and also the more frequent finding of KRAS mutations in AAH lesions than in associated adenocarcinomas. Frequent methods of achieving this state are by over expression of Myc protein or disruption of the Rb signaling pathway. The deregulation of mitochondrial (intrinsic) pathway of apoptosis may be studied by the expression of two genes: Bax and Bcl2. Bax is an apoptotic gene whose dimeric protein product formation induces apoptosis. In contrast, Bcl2 is a survival (antiapoptotic) gene and the dimer Bax/Bcl2 induces a neutralization of Bax and a loss of apoptosis. Bax/Bcl2 deregulation, as the inversion of the Bax:Bcl2 ratio, has been shown in preneoplastic lesions. A ratio less than 1 indicates hyperexpression of Bcl2 and loss of Bax, as compared with normal bronchial epithelium, has been shown to increase with the severity of the preneoplastic lesions from low grade to high grade lesions [50].

8.4. Limitless replicative potential

Normal cells have a limited replicative capacity, with the development of cellular senescence after a modest number of divisions. A major reason for cellular senescence and death is due to telomere shortening – about 50 to 100 bp of the telomeres are lost during each cell cycle. Telomere length can be maintained by the telomerase complex. The telomerase complex allows telomere length maintenance, which is required for an unlimited cellular proliferation [51]. Telomerase is present (usually in low levels, in normal human somatic cells (mainly stem cells), which are characterized by a definite proliferation potential. Restoring telomerase activity in normal somatic cells can indefinitely prolong cellular life span. However, the vast majority of tumors (around 90%), including lung cancers [52], express telomerase. In preinvasive bronchial lesions, hTERT mRNA

significantly increases along with the severity of the grade of the lesion [53] (Fig. 4). The level increases at the level of moderate dysplasia to culminate in severe dysplasia/*in situ* carcinoma and is correlated with P53 accumulation and resistance to apoptosis, featuring an immortal post M2 stage. It is preceded by telomere shortening, which occurs as early as squamous metaplasia, representing thus an earlier abnormality in bronchial carcinogenesis.

8.5. Sustained angiogenesis

Without the oxygen and nutrients supplied by vasculature system, tumors cannot achieve growth beyond a relatively small size. During tumorigenesis, cancers must acquire the ability to move from vascular quiescence to active angiogenesis the “angiogenic switch” [54]. VEGF with the VEGF-R co-receptors Neuropilin 1 (Np1) and 2 (Np2) are overexpressed in the majority of lung cancer [55,56]. A progressive increase of VEGF as well as Np1 and 2 expression was shown in the cells in preinvasive lesions of increasing grade [Lantuejoul, 2003 #8415], as compared to normal bronchial cells. This autocrine loop activation between VEGF and its receptors on premalignant tumor cells suggests other roles for VEGF signaling than angiogenesis. Loss or delocalization of semaphorin, a competitive ligand of VEGF and VEGF-R2, suggested a role of this autocrine loop invasion or migration.

8.6. Tissue invasion and metastasis

A characteristic (“hallmark”) of malignant tumors is the ability to invade the surrounding tissues and to metastasize. This is not an intrinsic feature at tumor conception, and *in situ* cancer cells closely resemble their invasive counterparts. The ability to invade and spread requires loss of cell to cell contact, the ability to traverse through a hostile environment (the stroma), the ability to penetrate basement membrane and enter blood vessels, and finally, the ability to survive and multiply in a foreign environment (metastases). Acquisition of these characteristics is often a late event, and tumors may demonstrate considerable heterogeneity in their expression. Acquisition of these properties may require tumor cell-stromal interactions. Thus, this is one hallmark that is not well represented in the preinvasive cell. As this chapter is about preneoplasia and preinvasion, tissue invasion and metastasis are not further discussed.

8.7. Genomic instability

In normal cells the rates of spontaneous mutations are low and for many years, it was considered that cancers could only harbor a few or modest number of driver mutations. However, most cancers contain numerous driver and passenger mutations [4,57]. In addition, multiple cytogenetic alterations including allelic losses at multiple gene loci [24,58] or polysomy [59] can be detected in the respiratory epithelium of smokers. Such findings can only be explained by the appearance of a mutator phenotype (i.e., the mutation rate in the cancer cells is much greater than that in normal cells) resulting in higher than background mutation rates than can be explained by rapid cell proliferation [57]. While the initial driver mutations may be due to exposure to tobacco or other environmental carcinogens, subsequent ones (and many passenger mutations) are the result of the mutator phenotype. Further evidence that the mutator phenotype appears in preneoplasia is the presence of aneuploidy, which is a reflection of widespread genetic and chromosomal abnormalities. Aneuploidy is frequently present in preneoplastic lesions and with both central and peripheral tumors, aneuploid preneoplastic lesions are more frequent in the peripheral compartment than in the central bronchi [60]. The presence of numerous tiny molecular clones and subclones in tobacco damaged respiratory epithelium may be regarded as further evidence of genomic instability [61]. It should be stressed that genomic instability is not regarded as a cancer hallmark. Rather, genomic instability is a result of the appearance of the cancer hallmarks during cancer progression. Thus genomic instability has been

labeled an enabling characteristic of cancer progression rather than an acquired capability (i.e. the hallmarks) [28].

9. Model systems for the study of lung preneoplasia

Understanding lung preneoplasia has been limited by a shortage of adequate sequential samples. Our group has immortalized human bronchial epithelial cells (HBECs) which can be manipulated by the introduction of oncogenes or by inactivating tumor suppressor genes [62][Gazdar, 2010 #15170]. By using combinations of such molecular manipulations, models for the premalignant, preinvasive, and invasive stages of lung cancer can be obtained and studied. Because the cells have stem cell like properties, they are pluripotent and can form squamous, adeno or undifferentiated (large cell) carcinomas. More recently our collaborators (Boning Gao and John Minna) have immortalized human small airway cells (HSAECs). These cells also have stem cell like properties and can be made to differentiate along the lines of peripheral airway cells – Clara cells, type 1 and type 2 pneumocytes.

10. Summary

Most lung cancers arise as a result of exposure to tobacco carcinogens, and there is a strong dose relationship between the extent of exposure and the subsequent development of lung cancer, often several years or decades after smoking commencement. Preneoplastic and preinvasive lesions develop during this lengthy period, and many of the molecular changes present in tumors can be detected in preinvasive lesions and even in histologically normal epithelium. Because of the difficulties of sampling the peripheral airways, most of our knowledge of preneoplasia is derived from a study of the central airway epithelium. However, the advent of CT guided screening approaches have resulted in increased recognition and laboratory study of the putative precursor lesion for peripheral adenocarcinomas, namely atypical adenomatous hyperplasias. Smoke exposure results in extensive damage to the entire respiratory epithelium with the appearance of multiple foci of molecularly damaged and histologically altered lesions (field cancerization). Both field cancerization and intrapulmonary spread or metastases may result in patients having multifocal cancers, although the survival of patients with such multifocal cancers (in the absence of distant metastases) is surprisingly favorable. Lung cancers arising in lifetime never smokers have different clinical, pathological and molecular differences from those arising in ever smokers. Tumors in never smokers (mainly adenocarcinomas) have a high percentage of EGFR gene mutations and demonstrate a much more restrictive field effect than tumors arising in smokers.

Six hallmarks of cancer have been recognized: 1) self sufficiency in growth signals; 2) insensitivity to antigrowth signals; 3) evasion of apoptosis; 4) limitless replicative potential; 5) sustained angiogenesis; and 6) tumor invasion and metastasis. Except for the last hallmark (which is a characteristic of invasive cancers, as opposed to preneoplastic lesions) examples of all of the other hallmark features have been documented in preneoplastic and preinvasive lesions, both of the central and peripheral airways. These hallmarks result in the appearance of genomic instability which appears at the dysplastic stage and drives cancer progression with the appearance of numerous further driver and passenger mutations.

Recent advances in molecular biology, imaging techniques and pathology have greatly advanced our knowledge of the pathogenesis of lung cancers via a study of the multistage pathogenesis of lung cancers and have provided evidence for the concept of central and peripheral compartments of the lung and the tumors derived from them.

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Fig. 1.

Preinvasive changes in the bronchial epithelium of smokers: (A) Normal bronchial epithelium constituted of basal cells, ciliated cells and goblet cells. (B) Mild dysplasia: cell atypia and architectural distortion at the lower one third of a squamous metaplastic epithelium. (C) Severe dysplasia: increased thickness and cytological atypia on full thickness of epithelium. (D) Carcinoma *in situ*: cyto-architectural atypia and lack of progressive maturation and orientation from basal to upper layer of epithelium.

Fig. 2.
Atypical adenomatous hyperplasia without (A) or with (B) alveolar wall fibrosis.

Fig. 3. Expression of cyclins in carcinoma *in situ* (A) and moderate dysplasia (B): (A) Cyclin D1 over expression showing nuclear staining of the majority of cells in a carcinoma *in situ* (3A) and a case of moderate dysplasia (3B). (B) Cyclin E over expression showing nuclear staining of 70% of cells in a case of moderate dysplasia.

Fig. 4. Expression of apoptosis related proteins in bronchial preneoplasia: (A) Bax cytoplasmic staining in a normal bronchial epithelium. (B) Loss of Bax staining in a severe dysplasia with retention of Bax expression in normal epithelium cells on the surface. (C) Bcl2 staining of basal cells in normal bronchial epithelium. (D) Over expression of Bcl2 in a carcinoma *in situ* with a usual pattern of low expression on the normal epithelium.

Fig. 5. hTERT mRNA *in situ* hybridization in bronchial preneoplasia. (A) Low level of expression in normal bronchial epithelium. (B) High level of expression in carcinoma *in situ*.