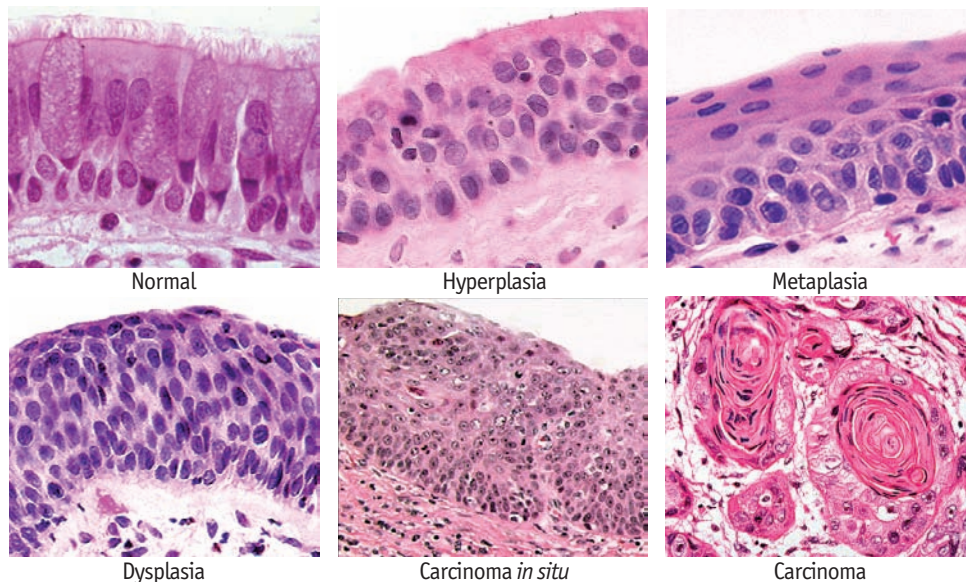


# The pathogenesis of pre-invasive lesion development



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## Provenance

Adapted from an ERS School Course.

## Competing interests

None declared.

## Educational aims

- ▶ To describe the progression of pre-invasive bronchial lesions.
- ▶ To highlight malignant transformation causes and risks.
- ▶ To explain somatic and epigenetic abnormalities contributing to lesions.

## Summary

The progression of pre-invasive bronchial lesions is a multifocal and multistep process. Morphological malignant transformation is driven by the accumulation of multiple genetic and epigenetic abnormalities, the rate and number of which determines the risk and rate of progression toward cancer. The field effect of mutagenesis in airways is characterised by the multifocality of the lesions. To date, no evidence has been presented for genetic alterations accumulating in a known sequence. No individual alteration is sufficient to predict the occurrence of invasive cancer in the bronchial tree.

Lung cancer is the leading cause of death from cancer worldwide. The high mortality rate (80–85% within 5 years) results, in part, from a lack of effective tools to diagnose the disease at an early stage. Given that cigarette smoke creates a field of injury throughout the

airways, epithelial malignancies, including bronchial epithelial malignancies, arise after a series of progressive pathological changes including hyperplasia, different grades of dysplasia and carcinoma *in situ* (CIS) [1]. These pre-malignant changes can accompany cancer

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or occur in the normal epithelium of individuals at high risk.

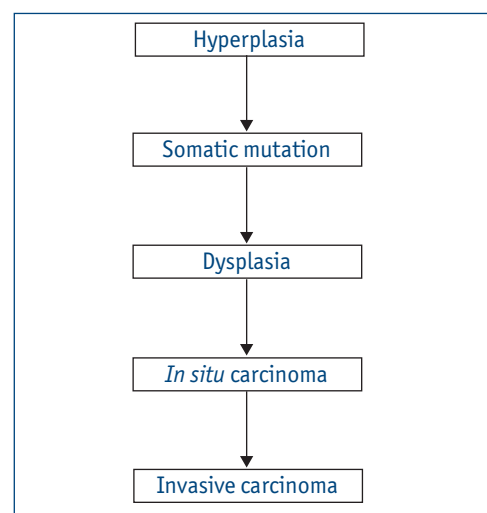
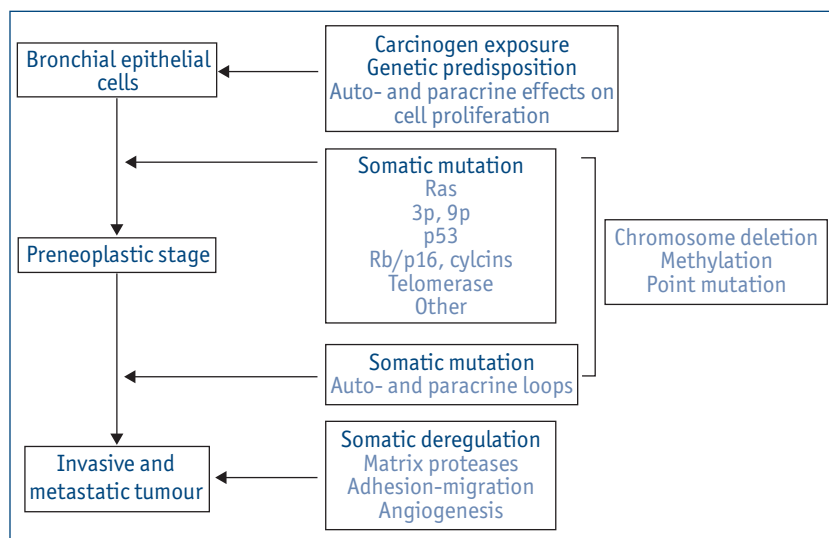
Approximately 85% of lung cancer cases are related to the use of tobacco [2]. Our knowledge of genetic predisposition for lung cancer has recently been expanded with the discovery of acetylcholine receptor genes (A3, A5 and B4), which have high-susceptibility variants for lung cancer. These acetylcholine receptors activate an autocrine loop driving proliferation and invasion at the level of bronchial epithelial cells. This is the first definite link between a polymorphism at a specific gene and pathogenesis [3, 4]. Other risk factors for lung cancer include chronic obstructive pulmonary disease and peripheral arterial disease.

## Development of invasive tumours

Bronchial epithelial cells undergo a long series of morphological changes to become invasive tumours (figure 1). During this transformation there is an accumulation of somatic mutations (chromosome deletion, methylation and point mutations) that disrupt key factors and pathways of cell proliferation and apoptosis. Somatic mutation starts before the pre-neoplastic stage, coinciding with transformations at the morphological level. During the pre-invasive stage, there is a strong involvement of autocrine and paracrine effects on cell proliferation in addition to other paracrine and autocrine loops taking place during the passage from CIS to invasion. At the primary invasive and metastatic stage, somatic deregulation causes changes in matrix proteases, adhesion-migration and angiogenesis.

The morphological steps from normal bronchial epithelium to carcinoma cells have been

**Figure 1**  
Steps of morphological changes for bronchial lesions.



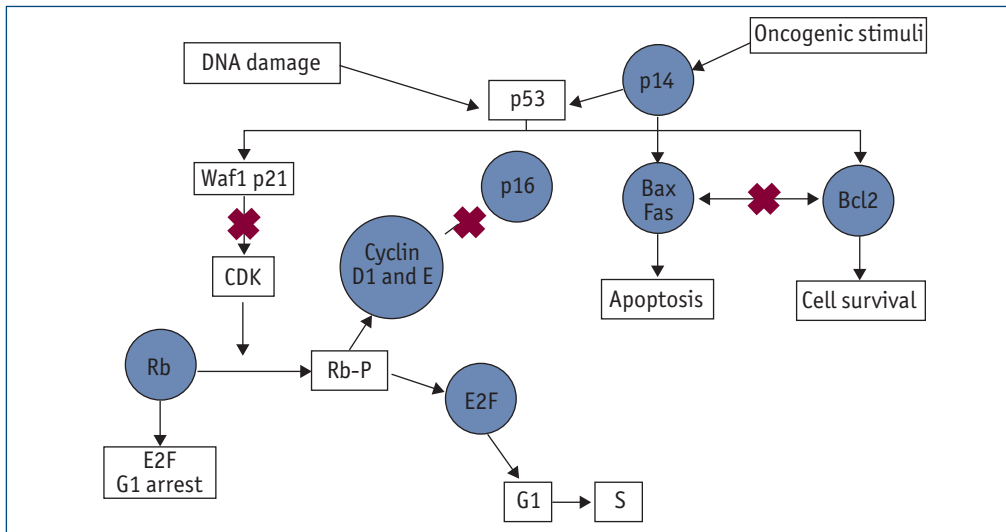
**Figure 2**  
Stages in the progression of bronchial lesions.

identified (figure 1). In normal epithelium, basal cells (the progenitor cells for squamous carcinoma and probably small cell carcinoma) can be seen, along with ciliated and goblet cells. In hyperplasia, polystratification of the basal cell line occurs. In metaplasia, a complete transformation of the glandular epithelium to squamous epithelium can be seen. There are mild, moderate and severe levels of dysplasia; in severe dysplasia, which directly precedes CIS, the orientation and maturation of cells is difficult to see. When the bronchial epithelium reaches the CIS stage it will almost certainly evolve into carcinoma.

With well-identified morphological stages of lesion progression, the main problem is predicting the progression from one morphological stage to the next. In total, <10% of metaplasias and <40% of mild or moderate dysplasias will become cancer. The only thing researchers are sure of is that CIS will lead to the development of an invasive carcinoma even with long-term local treatment. Therefore it is clear that molecular identity, cell-cycle regulation, apoptosis/senescence and DNA damage checkpoints are important factors to identify at all morphological stages in order to improve the prediction of pre-invasive lesions evolving into lung cancer.

## Field carcinogenesis

Sequential molecular abnormalities in the pathogenesis of epithelial bronchial tumours indicate that genetic changes commence in histologically normal epithelium, progress with increasing degrees of epithelial changes, follow a sequence from early to late stages, and are extensive and multifocal throughout the epithelium, indicating

**Figure 3**

The p53 pathway maintains genomic stability by arresting or eliminating damaged cells. For example, DNA damage activates p53 which turns on p21 transcription. p21 then binds to and inhibits cyclin-dependent kinases (CDKs), leading to hypophosphorylation of retinoblastoma protein (Rb). This prevents the release of E2F and blocks the G1 to S transition. p53 also acts through Bax and Fas to cause apoptosis and Bcl2 to halt cell survival.

a field effect known as “field cancerisation” [5]. Each lesion is independent from all others and has a multistep process of progression at the molecular level which depends on the number, accumulation rate and chronology of genetic alterations. The time sequence of progression differs from patient to patient.

Multiple clonal and subclonal patches of molecularly abnormal epithelial cells with or without morphological changes can be detected throughout the affected epithelium, specifically in smokers. Even histologically normal and *a fortiori* dysplastic lesions may show a number of somatic mutations of oncogene and tumour suppressor genes. Allelic loss (loss of heterozygosity, methylation of CpG islands and point mutations) of chromosomes 3p, 9p21 and 17p13 are common in smokers' epithelial cells [6, 7]. A p53 mutation is usually one of the first mutations to be seen. Promoter methylation is extremely frequent in smokers without cancer and occurs during very early metaplasia. Increased telomerase activity is also very frequent in smokers. Furthermore, genome-wide expression profiles that can detect cancerisation at a distance from the lesion have been identified [8].

Although the risk of progression increases from metaplasia to the successive grades of dysplasia and CIS, no clear prediction can be based on morphological grading alone. Knowledge of the molecular identity is necessary to evaluate the risk of progression.

## p53 pathway

The key pathway altered in pre-invasive bronchial lesions (excluding those preceding adenocarcinoma) is the p53 signalling pathway of cell cycle control and apoptosis (figure 3).

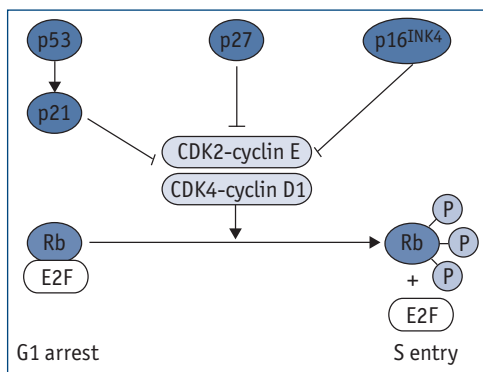
p53 is a transcription factor that acts as a sensor of stress responding to oncogenic stimuli, DNA damage and hypoxia. p53 transcription targets downstream genes involved in cell cycle arrest, apoptosis or DNA repair and upstream regulatory genes including p14 and mdm2. p53 is the most frequently mutated gene in lung cancer.

Stabilising p53 mutations are easy to detect in immunohistochemistry since the mutant protein has a long half-life, of up to 10 h, in contrast to the normal protein's half-life of 7 min. A p53 mutation may be seen as early as mild dysplasia. The presence of a p53 mutant phenotype is clearly linked to the progression of pre-invasion to invasion [9, 10].

## Rb pathway

A downstream signalling pathway of p53 involves the retinoblastoma protein, Rb, which plays a key role in mitochondrial apoptosis (figure 4). The Rb pathway mediates G1 arrest through the cyclin-dependent kinase (CDK) inhibitor p21. Unphosphorylated Rb binds to E2F1 and suppresses its transcriptional activity in G1-S transition. The protein complexes CDK6-cyclin E and CDK4-cyclin D1 phosphorylate Rb by releasing E2F1 during G1-S transition under the effect of growth factors or oncogenic stimuli. Therefore both the loss of and the hyperphosphorylation of the Rb protein disrupt the G1 checkpoint and are common in lung cancer.

The main causes of inactivation of Rb are through the overexpression of cyclin D1 or E or loss of p16 expression (figure 5) [10, 12]. Loss of p16 expression has been seen in 12% of moderate dysplasia and 30% CIS cases [13]. In patients with cancer, overexpression of cyclin D1 has been shown to occur in 6% of hyperplasia



## Bcl2 and Bax

In most small cell lung cancers (SCLC) Bcl2 is elevated in contrast to Bax which is downregulated in SCLC. This results in a Bcl2:Bax ratio of  $>1$  in 95% of SCLC making cells prone to

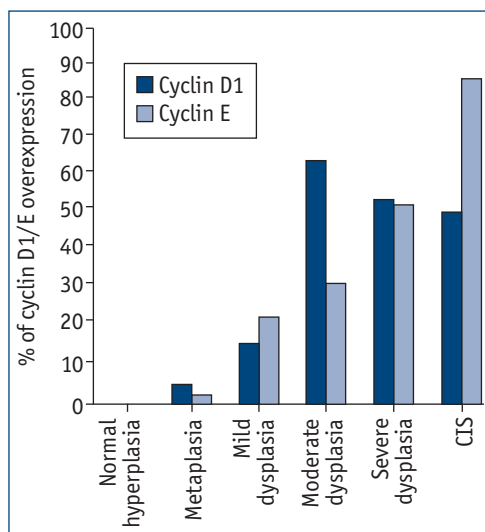
## Accumulation of abnormalities

# Telomerase

In pre-invasive bronchial lesions, hTERT expression is significantly increased in line with the severity of their grade [17, 18]. Nearly 100% of SCLCs have detectable or high levels of telomerase [19]. Telomere attrition reaches its maximum level at squamous metaplasia, as telomeres shorten following several cycles of all cell division, but further increase in telomere length as well as telomerase activation follows along the

**Figure 5**

*Cyclin D1 and E overexpression in pre-invasive bronchial lesions.*





progression from early to late pre-invasive lesions and invasion. Telomeres are the shortest length at the metaplasia stage, which is the initiation of cancer progression: after this point telomerase activation occurs encouraging tumour progression [17]. The squamous metaplasia phase may represent a M1 stage preceding p53/Rb inactivation and telomerase activation.

## DNA damage response

Commonly expressed markers of activated DNA damage have been seen in different stages of lung cancers. These include phosphorylated H2AX and CHK2. H2AX and CHK2 are key regulators in DNA damage checkpoints and the phosphorylation of these factors causes cells to enter into apoptosis. In normal bronchi, the level of phosphorylated H2AX is much lower than that seen in severe dysplasia, demonstrating that DNA damage is extremely high even in early precursor lesions [20, 21]. The level of phosphorylated H2AX increases steadily from normal bronchi to the CIS stage. Mutations in the DNA damage response pathway may also allow cell proliferation, genomic instability and tumour progression [20].

## Epigenetic modification

Epigenetic modifications are molecular mechanisms that regulate gene expression without changing the DNA sequence. These include the following. 1) The alteration of the methylation status of DNA within CpG islands leads to the silencing of tumour suppressor genes or the activation of oncogenes. 2) Covalent modifications of histone tails cause chromatin remodelling. 3) MicroRNAs lead to mRNA silencing and loss of protein expression.

### Aberrant methylation

DNA methylation is a normal process that occurs as part of a complex chromatin network influenced by modifications in histone structure. Changes in the pattern of DNA methylation have been shown to be a common event in human cancer and cause abnormalities of gene expression, chromosome structure, timing of DNA replication and chromatin organisation.

DNA methylation is restricted to "CpG islands" which, in expressed genes, are unmethylated. Aberrant methylation of CpG islands located near

the promoter region of these genes causes gene silencing. In lung cancer, as many as 100 genes have been found to be silenced by promoter methylation [22]. Methylation occurs in the early stages of lung cancer [23] and if it is detected in the sputum of smokers with dysplasia it has been shown to be a predictor of cancer development [24]. DNA methylation may also predict reoccurrence in stage I NSCLC [25]. A study examining methylation in eight genes in primary NSCLC and corresponding non-malignant tissue found that at least one gene was methylated in 85% of NSCLC [26]. These data demonstrate that methylation is the most common mechanism to inactivate cancer-related genes in NSCLC.

### Histone modifications

The acetylation and methylation of lysine residues on histone tails plays a crucial role in regulating chromatin packaging, nuclear architecture, gene expression and genomic stability [27–32]. In lung cancer cells, hypermethylation of CpG islands in promoter regions of tumour suppressor genes has been associated with particular combinations of histone markers. For example, deacetylation of histones H3 and H4, loss of trimethylation of histone H3 lysine 4 and an increase in trimethylated H3K9 and H3K27 have been seen in cancer cell lines.

Cancer cells have been demonstrated to have a loss of monoacetylated and trimethylated forms of histone H4 [32]. These changes in H4 occur early and accumulate as the tumour progresses. The data suggest that the global loss of monoacetylated and trimethylated forms of H4 is a hallmark of tumour cells in humans.

Epigenetic modifications of H4 have also been studied in 157 lung carcinomas using antibodies specific to lysines K5, K8, K12, K16 and trimethylated H4K20 [33]. Compared with normal lung, the cancer cells showed hyperacetylation of H4K5/H4K8, hypoacetylation of H4K12/H4K16 and a loss of H4K20 trimethylation. The loss of trimethylated H4K20 can be seen as early as pre-neoplastic bronchial lesion and is frequent in squamous cell carcinoma. The reduction increases with the grade of pre-invasive lesion. Therefore, H4K20 could be a potential candidate biomarker for the early detection of therapeutic approaches to lung cancer.

## Conclusion

A full array of molecular abnormalities leading to intrinsic proliferation, cell cycle check

points disruption, escape of apoptosis, cell immortalisation and epigenetic modification are the leading forces of progression of malignant transformation, particularly in smokers.

At any stage of the morphological scale, the number and accumulation of abnormalities of key signalling pathways represent predictive factors for invasion.

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