

Early detection of lung cancer: role of biomarkers

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Early detection of lung cancer: role of biomarkers. C. Brambilla, F. Fievet, M. Jeanmart, F. de Fraipont, S. Lantuejoul, V. Frappat, G. Ferretti, P.Y. Brichon, D. Moro-Sibilot. ©ERS Journals Ltd 2003.

ABSTRACT: Early detection of lung cancer requires none or few invasive techniques. Distal lung cancer (40% of the cases in most European countries) can be sensitively detected by spiral computed tomography. Theoretically, in 60% of cases, the proximal lesions (main to segmental bronchi, accessible by bronchoscopy) should be able to be detected by sputum cytology. Unfortunately, this very specific technique has a low sensitivity and is time consuming. Fluorescent bronchoscopy increases the detection rate of early or micro-invasive lesions and may be proposed in highly selected populations, but not as a screening test. Biomarkers in blood and sputum have not yet been clinically validated. However, the amount of data generated from studies first on resected tumours, then on early bronchial lesions and more recently on blood and sputum offer a wide field for investigation.

Lung carcinogenesis is a multistep process characterised by the accumulation of successive molecular genetic and epigenetic abnormalities, resulting in selection of clonal cells with uncontrolled growth capacities throughout the whole respiratory tract (field cancerisation). Molecular lesions far precede morphological transformation of preneoplastic bronchial lesions (dysplasia) or alveolar lesions (atypical alveolar hyperplasia). Genetic and epigenetic abnormalities in the genes involved in cell cycle, senescence, apoptosis, repair, differentiation and cell migration control may be detected on bronchial biopsies, on respiratory cells from the sputum and even in the circulating deoxyribonucleic acid (DNA). The key genes involved include those in the P53-retinoblastoma (Rb) pathways.

The balance between cyclin-dependent kinases and their inhibitors regulates the level of Rb phosphorylation and its function at G1-S transition; P53 plays at least two functions (cell cycle and apoptosis control). The balance of bax-bcl2 is important in the control of apoptosis as well as loss of fragile histidine triad expression. O(6)-methylguanine-DNA methyltransferase seems to be important in DNA repair control, the RAR β receptor in differentiation, and cadherin H and E and different metalloproteases genes in cell migration.

The demonstration of hyperexpression or silencing of these genes needs different validated techniques: immunohistochemistry on biopsies or cytological preparations, molecular biology techniques for mutations, loss of heterozygosity and aberrant methylation abnormalities. Automation and miniaturisation of these techniques will allow early detection and may be widely applied once clinically validated.

Eur Respir J 2003; 21: Suppl. 39, 36s–44s.

Lung cancer is the leading cause of cancer-related death, not only in males all around the world but also in females [1–3]. Despite the advances made in diagnosis and treatment in the last few decades, the prognosis of lung cancer is still very poor, with a 5-yr overall survival generally <10% in many countries. Lung cancer is detected by symptoms at a time when the local and moreover the metastasis extension hamper any hope of cure. Besides tobacco cessation and tobacco prevention, early detection of lung cancer is clearly the only way to improve the overall survival in the lung cancer epidemic. Detection of lung cancer-related antigens in the blood, such as carcino-embryonic antigen, failed to be of use in early detection. However, the amount of data generated from studies of oncogene

activation and the range of molecular and genetic abnormalities on resected tumours [4–7] has opened a wide field of investigation and most of the groups working on lung cancer are currently trying to validate accurate biomarkers for the early detection of lung cancer.

Multistep carcinogenesis and field cancerisation

Lung carcinogenesis is a multistep and multicentric process, characterised by the stepwise accumulation of genetic and molecular abnormalities after carcinogen exposure, resulting in the selection of clonal cells with uncontrolled growth capacities [8–11]. Molecular lesions

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Keywords: Biomarkers
early detection
fluorescent bronchoscopy
lung cancer
methylation
preneoplasia

Received: July 10 2002
Accepted after revision: August 22 2002

This group was supported by grants from DRC Grenoble, Equipe labellisée "La Ligue".

occur in normal looking epithelium in the absence of dysplasia. These have been shown to precede the morphological step of preneoplastic bronchial lesions, which are described as metaplasia, mild dysplasia, moderate dysplasia, severe dysplasia and carcinoma *in situ* (CIS) [12, 13]. As metaplasia in smokers displays genetic abnormalities, it can now be considered as a preneoplastic lesion. These lesions are multiple, reflecting the fact that the carcinogenic process may randomly affect any site in the bronchial tree and concomitant lesions can be of different ages and might progress at different rates towards invasion [14–17]. This typically represents the field cancerisation process defined previously. There is no definite prediction of delay of progression to invasion that can be deduced from the morphological grade, since even CIS may progress or regress. The current hypothesis is that molecular characteristics of any individual lesion, with regard to deregulation of cell cycle or apoptosis, might reflect its potential for progression [18–20]. However, most high-grade lesions are now treated locally, which means that their natural history cannot be clearly understood. Alternatively, the cumulative rate of molecular abnormalities at any random lesion, regardless of its morphological grade, might reflect the severity of the cancerisation field and be predictive of the susceptibility of an individual patient to develop a cancer in the bronchial tree (fig. 1). In the lung parenchyma, atypical alveolar dysplasia, considered as a preneoplastic lesion preceding bronchiolo-alveolar carcinoma, also exhibits genetic abnormality accumulation [21, 22].

The accumulation of genetic and molecular abnormalities leads to an uncontrolled growth of clonal cells and an increased ability of these cells to migrate, which characterises cancer progression, *i.e.* tumour growth and metastasis. Uncontrolled growth and migration of a cell can be summarised as the dysbalance between the activation of oncogenes, which drive the cell to multiply and migrate, and the inactivation of tumour suppressor genes (TSG) [18] (fig. 2). The activation of oncogenes may be due to genetic modification: mutation, amplification, chromosomal rearrangement or epigenetic modification such as hyperexpression. TSG inactivation may be due to genetic modification (mutation,

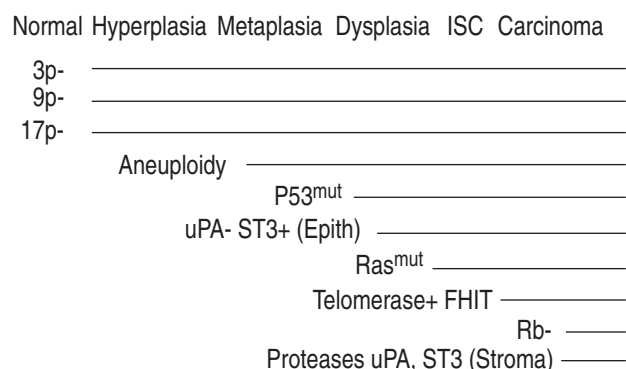


Fig. 1. –Chronology of genetic and molecular lesions. ISC: *in situ* carcinoma; uPA: urokinase type plasminogen activator; ST3: stromelysin 3; FHIT: fragile histidine triad; Rb: retinoblastoma; ^{mut}: mutation; +: activation; -: inactivation.

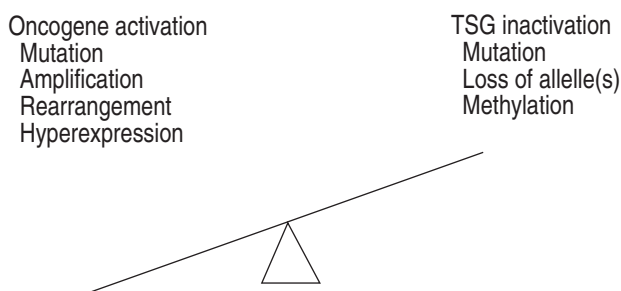


Fig. 2. –The involvement of oncogenes and tumour suppressor genes (TSG) in tumour growth and metastasis.

loss of chromosomal material (one or two alleles) or an epigenetic phenomenon such as methylation). Genetic modifications seem difficult to correct without a very well-targeted gene transfer. Epigenetic modifications seem more easily accessible to pharmacological therapy (demethylating agents). Therapeutic efforts are also directed at the result of a genetic modification (*e.g.* farnelysating agents to block the pathway of a mutated ras gene). Oncogenes are generally considered as dominant genes and TSG as recessive genes needing modification on both chromosomes to inactivate the gene. Whatever the mechanisms, the loss of expression or, on the contrary, the hyperexpression of a protein are the best methods to show the inactivation or the activation of a gene. All the methods of detection of malignant cells at the deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or protein level may be applied on different samples obtained from the patient (blood, sputum, biopsies). However, to be used as a biomarker they need to fulfil different requirements.

Mechanisms of genetic and molecular abnormalities: gene silencing and overexpression

During cellular division, the loss of DNA or chromosomal rearrangement increases with the rate of synthesis and division. It was shown several years ago, using cytogenetic techniques, that deletion of the short arm of chromosome 3 (3p) is very frequent in lung cancer. Like other chromosomal deletions, it corresponds to a site where one or several TSG are present and therefore inactivated. For a TSG previously identified, it is easy to check the deletion on the corresponding chromosomal site. In contrast, repetitive deletions at an unexpected chromosomal site indicate the need to seek a new putative TSG at this location by identifying mutations or methylations on the remaining allele. Due to the high density and the large size of the deletion on 3p the number of TSG(s) suspected at that location is high. Fragile histidine triad (FHIT) seems a good proposition at 3p14.2 [23].

Loss of chromosomal material not visible with classical cytogenetic techniques can also be shown. First, several probes can be used, which specifically complement a part of a pair of chromosomes in which a known or a putative TSG is located. After amplification of the DNA and electrophoretic migration,

the two bands corresponding to the maternal and paternal chromosomes are separated owing to the slight polymorphism of those two chromosomes. The heterozygosity of the gene visible in normal tissue, such as in lymphocytes from the blood, is then compared with its loss of allele on tumour cells where only one band is visible, demonstrating the loss of one allele or loss of heterozygosity (LOH). This loss is an early event frequently detected on different chromosomes (3p14, 9p21, 17p13, 13q14, 5q21, 1q, 2q, 8q) and they may be detected in histologically normal or metaplastic epithelium in smokers or exsmokers [19, 20, 23].

LOH can now be studied with miniaturised and automated techniques on microarray [24]. This allows a wider study of chromosome loci and a decrease in artefacts, due to the preparation of the sample using small sequences such as the single nucleotide polymorphism. The limit of these techniques is the need for a high ratio of neoplastic cells to avoid contamination by surrounding normal cells and the need for microdissection on samples such as bronchial biopsies. In sputum, separation of epithelial cells is probably necessary to avoid contamination by inflammatory cells. The number and frequency of LOH at specific gene loci have been clearly shown to increase with the severity of the bronchial preinvasive lesions. Homozygous deletion, that is the loss of the two alleles of a single gene, can be shown using the fluorescence *in situ* hybridisation technique with the simultaneous fixation of a gene-specific probe and a centromeric probe of the same chromosome [25]. It indicates the complete inactivation of the gene, but at the present time this technique is difficult to apply on a large scale as it is time consuming.

Aberrant gene methylation is a preferential method for gene silencing. One way to control the expression of a gene is through methylation of the cytosine phosphate guanosine rich regions (CpG islands) of the chromosome (fig. 3). Most of the chromosomes in a vertebrate are inactivated and the only unmethylated areas are those where the concentration of the CpG island is high and this corresponds to the promoters of the gene. An aberrant methylation in one of these areas induces a silencing of the gene. Tumours generally exhibit a global hypermethylation and a regional promoter hypermethylation that is associated with silencing of gene expression. The discovery of numerous hypermethylated promoter regions in TSGs along with a better understanding of gene silencing

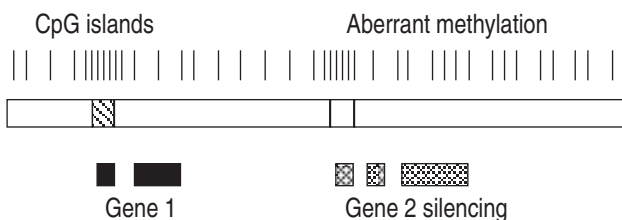


Fig. 3.—The aberrant methylation of cytosine phosphate guanosine rich regions (CpG sites) of the promoter induce a silencing of the gene expression. □: methylated CpG sites; ▨: nonmethylated CpG sites.

mechanisms indicates that DNA methylation is an alternative mechanism of inactivation of TSGs. In lung cancer [26–32], DNA methylation contributes to inactivation of numerous TSGs such as P16, P15, glutathione transferase 1, O(6)-methylguanine-DNA methyltransferase (O⁶-MGMT), tissue inhibitor of metalloprotease (TIMP)-3 and death associated protein (DAP)-kinase. Aberrant methylation can be shown using particular amplification techniques (methylation-specific polymerase chain reaction (PCR)). This technique seems promising not only on microdissected tissue but also on sputum and circulating DNA conserved in simple fixative. The increasing number of genes to study will probably require the use of methylation microarrays.

The use of microarrays in the search for mutations will be also necessary since classical sequencing or approaching techniques are difficult to apply on a large scale and the number of different possible mutations may be high (P53 for instance) [33]. Gene-specific or even lung cancer-specific microarrays are presently at different stages of realisation.

When antibodies directed against an oncogene or a TSG are available, the simplest way to evaluate the hyperexpression or the loss of expression of a gene is by immunohistochemistry [34, 35]. Differences in kinetics between wild or mutant proteins can be used to indicate a mutation in the gene (see below for P53). Since it is a sensitive *in-situ* technique, the number of advantages are high, particularly the comparison of the difference in expression within the histological lesions and the normal surrounding tissue, the change in subcellular compartment expression (nucleus, cytoplasm, membrane) and the use of internal control of protein expression (*e.g.* expression of a protein in blood vessels). The limits are the variable specificity of the antibodies, which needs cautious validation, and the ability of a protein to be detected on formalin fixed-samples and not only on frozen ones

Oncogenes and tumour suppressor genes

Different families of genes are implicated in the process of tumour growth and metastasis (fig. 4). The first family of genes involved, the growth factors and their receptors, are located at the cell membrane [36–39]. The epidermal growth factor receptor is overexpressed

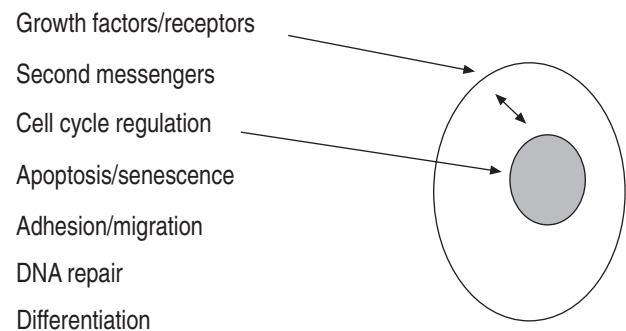


Fig. 4.—Different families of genes implicated in the process of tumour growth and metastasis. DNA: deoxyribonucleic acid.

in at least 80% of nonsmall cell lung cancers (NSCLC). Blocking agents, such as antibodies or new molecular agents, have a potential role in the chemopreventative treatment of such overexpressed growth factor receptors.

Second messengers allow transmission of the proliferation signal from the cell membrane to the nucleus. Of these second messengers, ras seems particularly useful for the early detection of adenocarcinoma [40–47]. Most ras mutations affect codon 12 of K-ras in 30% of adenocarcinomas in smokers and 5% in non-smokers. Interestingly, the K-ras mutation seems specific for cancer and precancerous states, since it has been described in severe alveolar dysplasia and atypical alveolar hyperplasia [21, 22]. The identification of a mutation in this gene in sputum, for example, could correlate with the development of cancer in the lung. The study of mutations in this gene appears to be interesting, since there are few mutations to study and the techniques are very sensitive. One cell with a ras mutant allele can be detected in a background of 10,000 cells with wild-type alleles [48].

The cell cycle regulation genes gather different gene networks that are involved in the progression of a dividing cell through successive of phases of the cell cycle. Some of these genes control the proliferation process at the different checkpoints of the cycle (G1-S, G2-M) and are prone to deregulation during the oncogenic process. One of the most important networks is the P53-retinoblastoma gene (Rb) pathway (fig. 5). Rb proteins, D-cyclins and cyclin-dependant kinase inhibitors belong to a group of genes of the internal cell cycle machinery that might be deregulated. The external machinery comprises P53, Mdm2 and Waf1 P21 proteins.

P53 has been called "guardian of the genome" [49, 50], as once it is activated, as a result of genotoxic stress, it reduces Rb phosphorylation and induces a stop at the G1-S check-point to allow DNA repair or to drive the cell to an apoptotic programme through bax/bcl2 and other P53-induced gene regulation processes. Inactivation of P53 leads to a loss of control of the G1 arrest and also to a loss to apoptosis leading to uncontrolled growth.

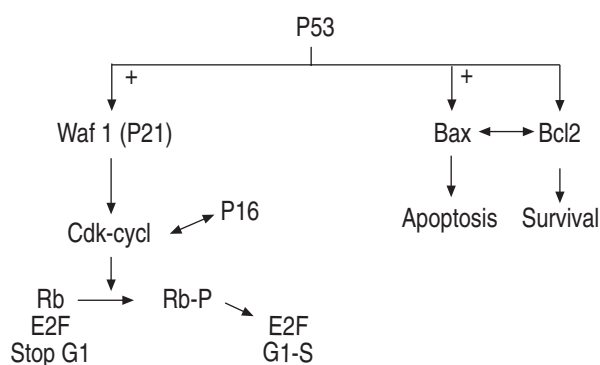


Fig. 5.—Signalling pathway of the P53 and retinoblastoma (Rb) gene, which controls the G1-S check point of the cell cycle and apoptosis. Bax, bcl2, Waf1 (P21), P16 and other cyclin-dependent kinases (cdk-cycl) are a part of the interregulated genes.

Mutation of P53 is very frequent in lung cancer and occurs in 50% of NSCLCs and 75% of small cell lung cancers (SCLCs) [51]. Most of the mutated proteins have an increase half life, with an accumulation of P53 protein in the nucleus that can be shown easily by immunohistochemistry. If accumulation of P53 protein in >20% of the cells is quite synonymous of a mutation [34], 20% of the mutations may affect nonsense codon or splicing sites with truncated messenger RNA. In these cases, sequencing techniques are necessary to identify the mutation [52, 53]. Nevertheless, immunohistochemical accumulation of P53 on a bronchial biopsy seems a very interesting biomarker. It is absent in metaplasia, and when present in dysplasia it seems well correlated with the presence or the outcome of a cancer [54] (fig. 6). At the present time, mutations of P53 may be studied on microarrays since the large number of mutations to be studied is one of the limits of ancillary techniques [55–63].

The Rb gene [64] is inactivated in >80% of high-grade neuroendocrine lung tumours, particularly SCLC [65–67], but it seems difficult to propose the study of this gene as a biomarker at the present time since preneoplastic lesions of these tumours are not known and Rb loss is present in only 10% of the preneoplastic lesions of squamous cancer. In contrast, P16 and cyclin-D1, which regulate Rb phosphorylation, are deregulated in 50% of NSCLC. The current authors have observed a loss of P16 protein expression at an increased rate in different preneoplastic lesions using immunochemistry on bronchial biopsies. This loss of expression was associated, or not, with a progressive overexpression of cyclin-D1 [68, 69]. The potential mechanisms for P16 inactivation, leading to P16 loss of protein expression, are homozygous deletion, methylation and mutation in decreasing order of frequency [25]. P16 seems to be an interesting biomarker when using immunochemistry on bronchial or lung biopsies, on cell preparations such as bronchial aspiration or sputum, or in circulating DNA from the blood. Gene silencing may be proved by the presence of aberrant methylation. Abnormalities on P19, alternative reading frame product of the INK4a, a common locus

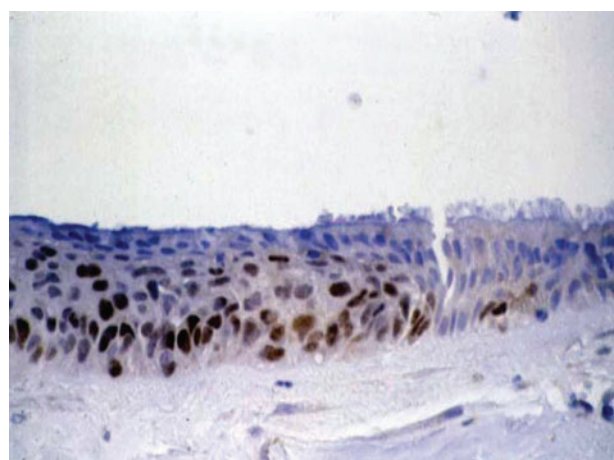


Fig. 6.—Nuclear accumulation of mutated P53 in bronchial dysplasia.

with P16 seems important in lung carcinogenesis [70, 71] but its role as a biomarker has to be evaluated.

The deregulation of apoptosis may be studied by the expression of two genes: *bax* and *bcl2* [72–74]. *Bax* is an apoptotic gene whose dimeric protein product formation induces apoptosis. On the contrary, *bcl2* is a survival (antiapoptotic) gene and the dimer *bax/bcl2* induces a neutralisation of *bax* and a loss of apoptosis. *Bax/bcl2* deregulation, as the inversion of the *bax/bcl2* ratio, has been studied in preneoplastic lesions [54]. A ratio <1 , indicating hyperexpression of *bcl2* and loss of *bax* as compared to normal bronchial epithelium, has been shown to increase with the severity of the preneoplastic lesions, from low grade to high grade. FHIT is also implicated in the apoptotic process; this gene is situated on 3p, which is frequently lost in lung cancer. Inactivation of this gene through LOH or loss of protein expression using immunochemistry has been shown as an early event in bronchial biopsies and was also found in sputum from smokers [75].

The senescence process is also deregulated in preneoplastic and neoplastic lesions. Telomeres are located at the end of all eukaryotic chromosomes [76, 77, 78] and are important in maintaining their stability. Foetal cells and some stem cells have telomerase activity to compensate for telomere shortening during replication (fig. 7), whereas the vast majority of normal adult cells do not exhibit telomerase activity. However, telomerase activity is detectable in most human cancers, particularly in SCLC and NSCLC. Telomerase activity may be also detected in precancerous lesions of the lung, reflecting the early involvement of the molecule in lung tumour genesis [79].

The four gene families mentioned above (growth factor/receptor, second messenger, cell cycle regulation, apoptosis/senescence families) are not the only ones involved in the process of tumour growth and metastasis, since adhesion/migration genes, DNA repair genes, differentiation genes and probably many others can be implicated in this complex process. At the present time, silencing or activation of different genes in these families have been proposed as biomarkers and the silencing of these genes through aberrant methylation is presently being studied on different samples, particularly in sputum and circulating DNA.

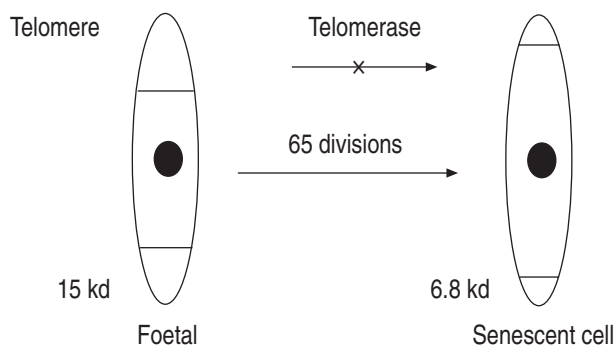


Fig. 7. – Telomerase, when activated, avoids shortening of the telomere, which leads to senescence.

What makes a good biomarker?

A good biomarker should require none or a minimally invasive technique of sampling [80]. Bronchial or lung biopsies are important to set up the value of one biomarker, but are difficult to propose for a screening programme. Bronchoalveolar lavage or brushings are less invasive but still require an endoscopy [81–83]. Sputum seems very interesting [84], but besides the problem of conservation or preparation, the main problem is the time necessary to collect good quality induced sputum (15–30 min with a specialised nurse or technician). However, a 3-day collection on Saccomano fixative seems to be good enough to study DNA but not RNA modifications (unpublished data). Circulating DNA in blood seems a very promising way to study biomarkers for early detection of lung cancer. Quantitative PCR only requires a very small amount of tumour DNA. Although the origin remains obscure, the presence of circulating tumour DNA is well established and could appear at the first stage of the disease before a metastasis process [85, 86].

A good biomarker must show a significant difference between the tumour and normal tissue and should correlate with cancer progression. To be applied in a screening programme, it should be able to be performed at a low cost and a high efficiency. Ideally, it should be used to target the treatment or to control its efficacy (intermediate end-point marker) once a chemoprevention programme has been proposed.

Clinical application

Spiral computed tomography (CT) is undoubtedly the best technique to detect small peripheral nodules early on. However, even if it is more accurate than chest radiography [87], spiral CT may not detect early proximal bronchial lesions. In the study of HENSCHKE *et al.* [87], only one proximal bronchial cancer was detected in a group of 27 cancers. Probably, the amelioration of the techniques will allow for better detection of these lesions. Sputum cytology is a highly specific technique, but unfortunately the sensitivity is very low. In the study of J. Jett (J. Jett, Mayo Clinic, Boston, MA, USA, personal communication), for example, only two cancers were detected on sputum cytology in 1,500 patients. As well as its lack of sensitivity, sputum cytology is also a time consuming technique. Automated cytology has been validated for cervical preparations, but even if different techniques are actually proposed, none have been validated on sputum [88].

The best way to view and biopsy proximal lesions from the trachea to most of the subsegmental bronchi is *via* fibreoptic bronchoscopy. This technique can also not be proposed as a screening technique since it is an invasive procedure that may not be cost effective unless it is applied to a highly selected population of patients. Fluorescent fibreoptic bronchoscopy clearly increases the sensitivity of detection of very early lesions. The first system developed by LAM *et al.* [14] was the Life System. Illumination with a determined wavelength (520 μm) laser light induces an auto

fluorescence of the bronchial epithelium, probably due to the reflection of the light on the inner part of the bronchial wall (collagen). When there is an increase of the thickness of the bronchial mucosa it appears as a dark brown area, corresponding to preneoplastic, micro-invasive or to inflammatory lesions of the bronchial wall. As this allows for a targeting of the biopsy it clearly increases the rate of detection of preneoplastic lesions. The relative sensitivity was multiplied by 4.7 for low-grade preneoplastic lesions and by 2.3 for high-grade lesions, as compared to white light bronchoscopy in a study of 278 patients recently published by the current authors [89]. However, the specificity of this technique is not very high (55% in the current authors' study). This study allows a better determination of the at risk population. The duration and intensity of tobacco consumption is correlated with a high rate of preneoplastic lesions but the most important factor is a history of squamous cancer. In the present authors' series, the 11 patients with lung cancer cured by surgical resection had a history of squamous cancer, no preneoplastic lesions were detected in the bronchi of patients with an history of adenocarcinoma. Fifty-five per cent of the patients had several high-grade lesions and in 40% of the cases, these lesions were bilateral. This clearly illustrates the theory of field cancerisation. There is no consensus at the present time on the way to manage these lesions. CIS seems to progress to invasive cancer in most cases, particularly after intraluminal treatment with electrocautery or photodynamic therapy [16, 17, 90]. Moderate and severe dysplasia are considered as preneoplastic lesions due to the risk of progression of these lesions or the accumulation of molecular abnormalities at their level and most of the groups consider that they require a local treatment with electrocautery. The discussion persists on metaplasia and mild dysplasia and a randomised trial is underway. However, it is very difficult to prove the natural history of these lesions and the role of treatment intervention. The new World Health Organization/International Association for the Study of Lung Cancer (IASLC) classification [13] gives the pathologist better elements to classify these lesions and the interobserver variability will probably decrease with training of the pathologists, as they were not really aware of these lesions up to now [91, 92]. The training and the interobserver variability will also ameliorate between the endoscopists using different types of fluorescent endoscopic devices [93]. The two main problems are the representativity and the reproducibility of the biopsies. When the number of biopsies increases from one to five on a bronchial division, the rate of finding more severe lesions, *e.g.* microinvasive carcinoma instead of CIS, increases as well. The repetition of biopsy on the same spot several months later is very difficult to interpret. The site of a preceding biopsy is easy to locate, owing to the fact that there is a dark spot under autofluorescence examination in the area of a previous biopsy. But when the new biopsy shows a worsening of the lesion, it is difficult to prove if it is really a worsening or a lack of sampling on the first biopsy. When the new biopsies fail to show preneoplastic lesions, either the

first biopsy removed all of them or it corresponds to amelioration or a real cure of the lesions. These two problems, in particular, obscure the natural history of these lesions and one must be cautious when interpreting the evolution of those during longitudinal follow-up of a given lesion.

Spiral CT and fluorescent bronchoscopy clearly increase the rate of detection of preneoplastic or early cancer. The role of the biomarkers, however, may be important either to select a population that would require radiological or endoscopic screening programmes or to recommend more aggressive diagnostic procedures or therapeutic interventions when radiological or endoscopic lesions have been found. The present authors recently showed that the number of molecular abnormalities on bronchial preneoplastic lesions correlates with an event of cancer [55]. To evaluate the role of these biomarkers two different protocols were initiated as follows: Biomarkscan, a pilot nonrandomised early detection programme in very high-risk patients; and Depiscan, part of the French screening randomised control to trial in asymptomatic smokers.

In the Biomarkscan programme the patient will have spiral CT and induced sputum and blood sampling every year, for 5 yrs. A fluorescent bronchoscopy will be performed every 2 yrs for the patients with an history of squamous cancer and at the beginning for the group of symptomatic smokers, and repeated after 3 or 6 months when a high-grade or low-grade lesion is found. All the patients and the volunteers recruited in the control group will have a questionnaire about respiratory symptoms and tobacco consumption and tobacco cessation will be recommended. The patients in the first group will include those with an history of lung or upper airways cancer cured by surgery; in the second group, symptomatic smokers; in the third group, patients with a cancer at the time of inclusion in the protocol (this will be used as a positive control group for the determination and the follow-up of biomarkers in blood and sputum); and the last group, normal nonsmoking volunteers who will be used as a negative control group for the biomarkers. The aims of these protocols are to increase early detection using spiral CT and biomarkers, to identify biomarkers predictive of irreversibility and progression in high-grade preneoplastic lesions, to draw a molecular and genetic identity card to assess the risk of cancer and, finally, to establish a phenotypic receptor characterisation in order to set up a chemoprevention programme. A very preliminary result of this programme was the detection of 23 aberrant methylations in the promoters of six TSG genes (P16, FHIT, DAP-kinase, O⁶-MGMT, TIMP-3, E-cadherin) in the bronchial aspiration of the first 76 patients. Four patients had two methylations of these genes and five patients had methylation of at least one gene without cancer at the time of endoscopy.

The Depiscan programme is a national randomised programme to assess the value of spiral CT in the reduction of specific mortality of lung cancer. The patients are not the same since they are asymptomatic smokers. In those patients, blood and sputum sampling

will be performed as well as repeated spiral CT to assess the modification of the biomarkers. Those two protocols are included in the international collaboration driven by the European Lung Cancer Early Detection (EULCED) Group [81, 82].

Conclusion

Clearly, the future of the use of biomarkers will have to go through miniaturised and automatic techniques, probably using microarrays. Unfortunately, in lung cancer there are no sensitive and specific biomarkers, such as prostate specific antigen in prostate cancer. Several biomarkers will probably have to be used together, including P53, ras and the methylation of different genes. This will increase the cost of using biomarkers; however the huge amount of money generated by tobacco use could be better directed towards benefiting smokers in this way. Conversely, even if early detection techniques seem promising, it would be unethical for physicians to not concentrate on the only way to reduce the incidence of lung cancer, which is smoking cessation programmes and smoking prevention programmes.

Acknowledgements. The authors would like to thank M. Lorinet and I. Berruyer for typing the manuscript.

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