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Detection of early lung cancer lesions in surgical resections and in bronchial and transbronchial biopsies

Odkrivanje zgodnjih oblik pljučnega raka v kirurških resektatih in bronhialnih in transbronhialnih biopsijah

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Background. *Overall bad prognosis of lung cancer is mostly due to too late detection of early lung cancer, which may be treated with good success. Therefore, different diagnostic methods are developing for more efficient detection of early lung cancer: besides modern radiological, bronchoscopic methods with additional fluorescence techniques, quantitative cytological investigations, also histological and molecular investigations are included. Histology may reveal early preinvasive lung cancer lesions, associated early during multistep lung carcinogenesis with molecular genetic changes.*

Patients and methods. *Preinvasive epithelial lung cancer lesions we searched in two groups of patients. In the first group of 316 patients from the period March 2003 – August 2006, 498 bronchial and transbronchial biopsies were examined for squamous metaplasia and dysplasia, carcinoma in situ, and invasive tumours. In the second group of 238 patients from the period January 2004 - August 2006, resected primary lung tumours were analysed for preinvasive and invasive neuroendocrine tumours and atypical adenomatous hyperplasia.*

Results. *The most frequent changes in bronchial and transbronchial biopsies were squamous metaplasia (46.5%), simple or goblet cell hyperplasia of the bronchial epithelium (44.3%), malignant tumours (20.66%) and squamous dysplasia (16.1%), but rare carcinoma in situ (0.63%).*

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia was found in 15 (6.3%) cases in the vicinity of 238 resected lung cancer specimens, carcinoid in 12 patients (5%), and mostly combined large cell neuroendocrine cancer in 21 patients (8.8%). Atypical adenomatous hyperplasia was found in 2 patients.

Conclusions. *Classical histological analysis should be focused on detection of early preinvasive epithelial lung cancer lesions. Additional available molecular investigations may reveal gradual genetic changes characteristic for a series of the preinvasive epithelial histological changes. More systematically oriented sampling of lung resections could increase the incidence of the lesions, arising from pulmonary neuroendocrine cells, and especially the incidence of atypical adenomatous hyperplasia.*

Key words: lung neoplasms – pathology; metaplasia; hyperplasia; carcinoma in situ; biopsy; preinvasive epithelial lesions; light-microscopy

Izhodišča. *V celoti slaba napoved preživetja pljučnega raka je v večji meri posledica poznega odkrivanja te bolezni, saj le zgodnje oblike lahko uspešno zdravimo. Zato danes razvijamo različne diagnostične metode, s katerimi bi lahko uspešneje odkrivali zgodnje oblike pljučnega rak: Poleg modernih radioloških, novejših bronhoskopskih z dodatnimi fluorescenčnimi metodami, kvantitativnih citoloških preiskav tudi histološke in molekularne. Histologija lahko odkrije predinvazivne oblike pljučnega raka, ki jih že zgodaj na določenih stopnjah kancerogeneze spremljajo določene molekularne oziroma genetske spremembe.*

Bolniki in metode*. Predinvazivne epitelne oblike pljučnega raka so iskali pri dveh skupinah bolnikov. V prvi skupini 316 bolnikov iz obdobja od marca 2003 do avgusta 2006 so v 498 bronhialnih in transbronhialnih biopsijah iskali predvsem ploščatocelično metaplazijo in displazijo, carcinoma in situ in invazivne tumorje. V drugi skupini 238 bolnikov iz obdobja od januarja 2004 do avgusta 2006, ki so jim kirurško odstranili primarne pljučne tumorje, pa so iskali predvsem predinvazivne in invazivne nevroendokrine tumorje in atipično adenomatozno hiperplazijo.*

Rezultati*. Najpogostejše spremembe v bronhialnih in transbronhialnih biopsijah so bile ploščatocelična metaplazija (46,5%), enostavna ali mukocelularna hiperplazija bronhialnega epitelija (44,3%), maligni tumorji (20,66%) in displazija (16,1%), redko je bil odkrit carcinoma in situ (0,63%).*

V pljučnih resektatih je bila difuzna idiopatska hiperplazija pljučnih nevroendokrinih celic odkrita pri 15 (6,3%), karcinoid pri 12 (5%) in pretežno kombinirani velikocelični nevroendokrini karcinom pri 21 bolnikih (8,8%). Atipična adenomatozna hiperplazija je bila prisotna pri 2 bolnikih.

Zaključki*. Klasični histološki pregled mora biti osredotočen na odkrivanje zgodnjih predinvazivnih epitelnih oblik pljučnega raka. Dodatne razpoložljive molekularne preiskave pa lahko odkrivajo postopne genetske spremembe, ki so značilne za postopne predinvazivne histološke spremembe v epiteliju. Bolj usmerjeno vzorčenje resektatov bi verjetno povečalo incidenco patoloških sprememb, ki izvirajo iz nevroendokrinih celic, predvsem pa incidenco atipične adenomatozne hiperplazije.*

Ključne besede: pljuča novotvorbe – patologija; metaplazija; hiperplazija; karcinom in situ; biopsija; predinvazivne epitelne spremembe; svetlobna mikroskopija

Introduction

Lung cancer is the most important cancer in men and one of the most frequent also in women. Among all the patients with cancer, it is the leading cause of death, and it exceeds the sum of deaths due to cancer of breast, colon, and prostate.[1] The survival rate is still very poor, mostly because of too late detection of the cancer in advanced stages, in more than two thirds of cases. But on the other hand, Stage I patients may have a 5-year survival approaching 70%.[1,2]

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Therefore, the extreme effort is dedicated to its early detection by more complicated radiological, bronchoscopical, cytological, and molecular investigations. And finally, the classical histological analysis of diagnostic specimens and surgical resections should concentrate on the detection of precancerous preinvasive epithelial lesions, defined also by the last WHO classification of the lung tumours: squamous dysplasia, carcinoma *in situ*, diffuse idiopathic pulmonary neuroendocrine cell hyperplasia, and atypical adenomatous hyperplasia.[3] Therefore, the aim of the study was to find out the incidence of preinvasive lesions in our routine diagnostic bronchial and transbronchial biopsies and in surgical specimens.

Table 1. Histological findings in 316 patients with 498 bronchial and transbronchial specimens

Histological finding	Number of patients	Number of specimens
EPITHELIAL HYPERPLASIA	140 (44.3%)	191 (38.3%)
SIMPLE HYPERPLASIA	87 (27.5%)	101 (20.3%)
GOBLET CELL HYPERPLASIA	73 (23.1%)	90 (18%)
SQUAMOUS METAPLASIA	147 (46.5%)	188 (37.7%)
ANGIOGENIC SQUAMOUS DYSPLASIA (MICROPAPILLOMATOSIS)	3 (0.95%)	3 (0.6%)
BRONCHIAL EPITHELIAL DYSPLASIA	51 (16.1%)	59 (11.8%)
CARCINOMA *IN SITU*	2 (0.63%)	3 (0.6%)
BENIGN TUMOURS	4 (1.26%)	4 (0.8%)
INVASIVE CARCINOMAS	66 (20.88%)	78 (15.7%)
SQUAMOUS CARCINOMA	16	
ADENOCARCINOMA	16	
LARGE CELL CARCINOMA	15	
SMALL CELL CARCINOMA	12	
CARCINOID	1	
NOT SPECIFIED	1	
SUSPECTED MALIGNANCY	5	
NOT DIAGNOSTIC TISSUE	32 (10.12%)	38 (7.6%)

Patients and methods

There were 498 bronchial and transbronchial biopsies performed in 316 patients. The biopsies represent a part of random diagnostic biopsies from the period March 2003 – August 2006. The patients demonstrated various mostly not specific symptoms and signs of lung involvement because of some not yet determined pathological process. Standard routine stains of the histological slices included haematoxylin and eosin, van Gieson-Weigert staining for elastin and collagen, alcian blue staining for eventual mucinogenesis and Perls' staining to demonstrate haemosiderin deposition. The specimens were thoroughly examined for changes especially in the bronchial epithelium and in associated lung tissue.

Moreover, 238 patients with primary lung tumours, from the period january 2004-august 2006, are included in the study. They were treated surgically with lobectomy, bilobectomy or even pulmectomy together with the resection of regional lymph-nodes. Resected lobes or lungs with pulmonary tumour were sampled for the further histological examination. Besides routine hematoxylin and eosin stain, combined van Gieson-Weigert staining, alcian blue staining, some additional immunehistological stainings were employed. In all cases, marker for chromogranin A was used to demonstrate eventual neuroendocrine component of the tumour, including hyperplasia of neuroendocrine cells in the bronchial walls in the vicinity of the tumours.

In majority of cases, thyroid transcription factor 1 (TTF-1) was used to confirm

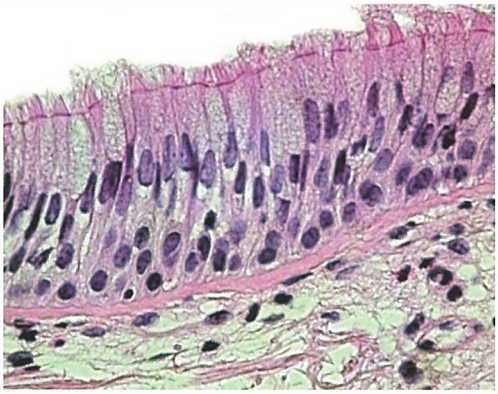

Figure 1. Simple epithelial hyperplasia with pseudostratification, haematoxylin and eosin.

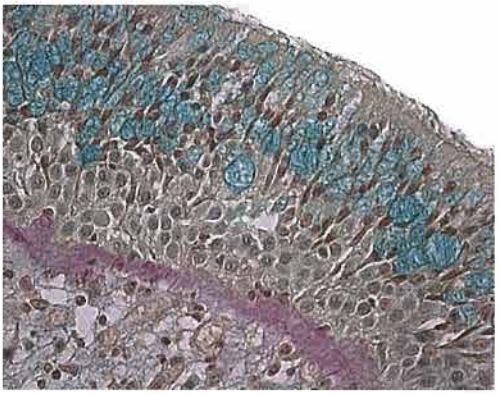

Figure 2. Combined superficial goblet cell hyperplasia and deep early squamous metaplasia, alcian blue.

most probable pulmonary origin of adenocarcinomas. In some cases, other additional immune-histological methods were used.

Results

Bronchial and transbronchial biopsies

Bronchial and transbronchial biopsies displayed a wide spectrum of histological changes in bronchial epithelium presented in Table 1. Individual changes may occur simultaneously, especially if the tissue specimen is abundant; in such cases, simple epithelial hyperplasia (with pseudostratification) is associated with goblet cell hyperplasia, focal squamous metaplasia and even squamous dysplasia.

Insufficient or inappropriate specimens presented mostly bronchial fibrous tissue with inflammatory changes, without any epithelium. Dilated mucous glands and ducts are the changes suggesting chronic catarrhal bronchitis. In the lung, there is only focal fibrosis or not specific inflammatory changes, without any bronchiolar or alveolar epithelium. Such unsatisfactory biopsies occurred in 32 patients, with mostly only one tissue specimen.

Epithelial hyperplasia was presented by focal or diffuse *simple hyperplasia* (Figure 1), or by focal or diffuse *goblet cell hyperplasia*.

Table 2. Interrelations between simple epithelial hyperplasia, squamous metaplasia and 59 cases of dysplasia.

	DIFFUSE SQUAMOUS METAPLASIA	FOCAL SQUAMOUS METAPLASIA	DIFFUSE SIMPLE HYPERPLASIA	OTHER ASSOCIATED LESIONS WITH DYSPLASIA
DYSPLASIA I	26 patients	11 patients	2 patients	2 (1 fibrosis and 1 adenocarcinoma)
DYSPLASIA II	6 patients	8 patients	1 patient	1 bronchial fibrosis
DYSPLASIA III	0	1 patient	0	1 carcinoma *in situ*

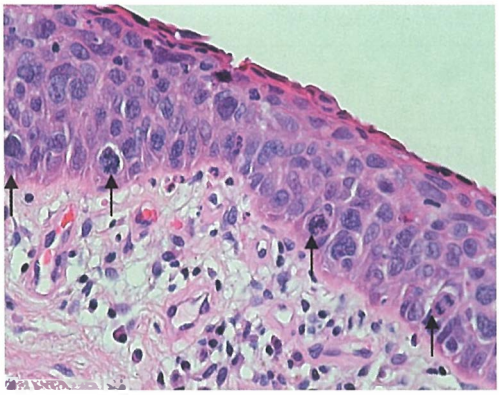

Figure 3. Moderate squamous dysplasia with numerous mitoses in the lower part of the epithelium, haematoxylin and eosin.

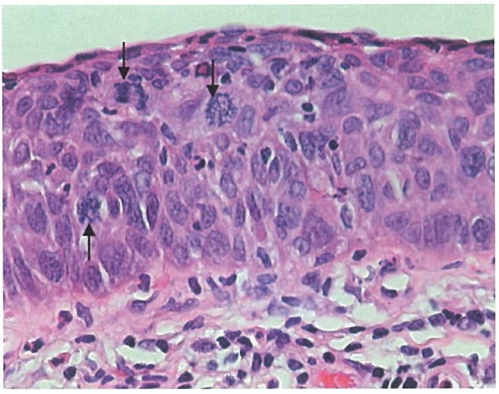

Figure 4. High-grade squamous metaplasia with mitoses in the upper and lower thirds of epithelium, haematoxylin and eosin.

In many cases, the latter may cover underlying *early squamous hyperplasia* represented by cells with obvious intercellular bridges (Figure 2). *Developed squamous metaplasia* affects the whole width of epithelium, and may be focal or diffuse, with hyperplastic or also with atrophic epithelium.

Dysplastic changes are mostly associated with developed focal or diffuse squamous metaplasia, but only in rare cases with simple epithelial hyperplasia (Figures 3, 4). They may be found in the vicinity of an intraepithelial or invasive carcinoma (Table 2).

A special form of relatively rare but unfavourable preinvasive dysplastic lesions is *angiogenic squamous dysplasia*, with characteristic vascular budding into epithelium, previously reported as micropapillomatosis (Figure 5).

Benign tumours included 2 bronchial squamous papillomas, endobronchial hamartoma and submucous leiomyoma.

The relative incidence of *malignant tumours* is such as expected, with predominance of squamous and adenocarcinoma, followed by small cell carcinoma. A relatively high incidence of large cell carcinoma is mostly due to insufficient and also damaged tumorous tissue. Suspected malignancies were characterized by keratin masses

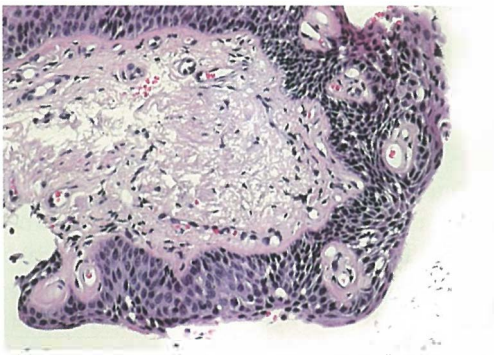

Figure 5. Angiogenic squamous dysplasia "micropapillomatosis" with vascular budding in the epithelium, haematoxylin and eosin.

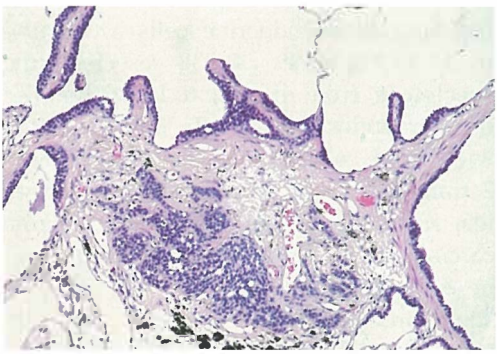

Figure 6. Discrete unrecognizable diffuse idiopathic pulmonary neuroendocrine cell hyperplasia in the epithelium and tumorlet, haematoxylin and eosin.

Table 3. Neuroendocrine (NE) lesions among 238 surgically resected lung specimens

Lesion	Number of patients and %
DIFFUSE IDIOPATHIC PULMONARY NE CELL HYPERPLASIA (DIPNEH)	15 (6.3%)
ASSOCIATED LESIONS	
DIPNEH + TUMORLET + CARCINOID	1
DIPNEH + TUMORLET + CARCINOID + BRONCHIOLO-ALVEOLAR ADENOCARCINOMA	1
DIPNEH + CARCINOID	2
DIPNEH + LARGE CELL NE CARCINOMA + PLEOMORPHIC CARCINOMA	1
DIPNEH + ADENOCARCINOMA	6
DIPNEH + SQUAMOUS CELL CARCINOMA (1 BIFOCAL)	3
DIPNEH + COMBINED ADENO-SQUAMOUS CARCINOMA	1
CARCINOID	12 (5%)
LARGE CELL NE CARCINOMA	21 (8.8%)
COMBINED LARGE CELL NE CARCINOMA	14 (5.9%)

without vital tissue, or by small nests of tumorous tissue, or by abundant necrotic masses with solitary cancer cells.

Surgical resections

Neuroendocrine lesions in surgical specimens are demonstrated in Table 3.

Diffuse idiopathic atypical hyperplasia of the lung neuroendocrine cells was found in 15 (6.3%) cases of 238 resected lung specimens, from discrete to larger nodules of neuroendocrine cells. It was associated with various neuroendocrine tumours, with 2 tumorlets (Figures 6, 7) with 3 carcinoids, and with 1 large cell neuroendocrine carcinoma, but also with not-neuroendocrine tumours, 6 adenocarcinomas (47%), 3 squamous carcinomas (one of them bifocal), and 1 combined adenosquamous carcinoma (which demanded pulmonary resections).

Carcinoid was found in 12 of 238 patients (5%).

Carcinomas with neuroendocrine component (in 2-100% of all tumour cells) were found in 21 of 238 patients (8.8%), out of them, there were 14 cases of combined large cell neuroendocrine cancer (with elements of squamous cell carcinoma or adenocarcinoma).

Only in 2 patients of 238 lung resections, *atypical alveolar hyperplasia* leading to peripheral adenocarcinoma was found at the periphery of papillary adenocarcinomas (Figure 8). Reactive cuboid metaplasia (pneumocyte II hyperplasia) may be found around almost all inflammatory and malignant neoplastic lesions, irrespective of carcinoma type. Cuboid metaplasia, together with endogenic lipid pneumonia, suggest tissue destruction, usually found in malignant tumours or necrotic inflammatora granulomatous lesions.

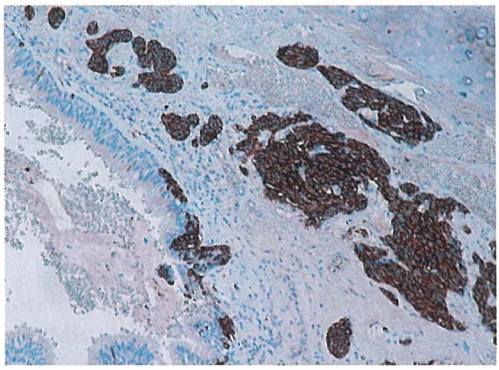

Figure 7. Small nests of hyperplastic epithelial neuroendocrine cells, presenting early diffuse idiopathic pulmonary neuroendocrine cell hyperplasia, and nests of tumorlet in bronchial wall, chromogranin A.

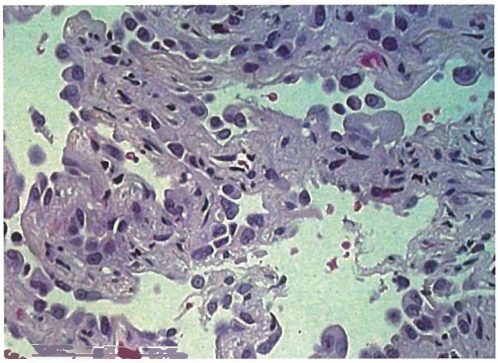

Figure 8. Atypical adenomatous hyperplasia, haematoxylin and eosin.

Discussion

The extreme effort is dedicated to an early detection of preinvasive or microinvasive lung cancer lesions by more complicated radiological examinations (e.g. low-dose spiral computer tomography),[4] autofluorescence bronchoscopy,[5] fluorescence bronchoscopy with the additional spectral analysis,[6,7] quantitative cytology[8,9] and molecular investigations.[10,11] And finally, also a classical histological analysis of diagnostic specimens and surgical resections should concentrate on the detection of precancerous preinvasive epithelial lesions, defined also by the last WHO classification of the lung tumours and mentioned in the introduction.[3]

Squamous metaplasia, dysplasia and intraepithelial cancer, leading to invasive squamous carcinoma, to large cell carcinoma, and to at least a great part of cases with pleomorphic cancer, are relatively frequent in patients with higher risk for the development of cancer (such as are also long-term smokers aged over 50 years).

Less frequent is diffuse idiopathic pulmonary neuroendocrine cells hyperplasia, leading to neuroendocrine tumours, such as are tumorlet, typical and atypical carcinoid, and probably to a part of large cell neuroendocrine carcinomas.

There is not always a sharp demarcation between reactive cuboid metaplasia and atypical adenomatous hyperplasia, therefore, the incidence of the latter is in a relative wide range.

Carcinogenesis of the lung cancer is a gradual multi-step process at both phenotypic and genetic levels, similar to those found in other organic systems. Histologic (phenotypic) preinvasive bronchial lesions are usually multifocal, and are preceded or accompanied by multi-step accumulation of molecular and genetic abnormalities. Molecular changes are very rare in normal epithelium, few in hyperplasia, but numerous in dysplasia and in carcinoma *in situ*.[12]

Ten to twenty molecular changes are associated with progressive reversible (hyperplasia, metaplasia, mild dysplasia) or irreversibile (severe dysplasia, carcinoma *in situ*, invasive carcinoma) histological changes (Table 4).

Early histological changes in bronchial and alveolar epithelium leading to lung carcinoma may be observed and followed, altogether with associated gradual genetic changes. The analysis of molecular gene-

Table 4. Chronology and frequency of genotypic and phenotypic-histologic changes during multi-step pathogenesis of squamous cell lung cancer (LOH– loss of heterozygosity, MSI-microsatellite instability, modified from Brambilla 2005)

Norma	hyperplasia	metaplasia	dysplasia	carcinoma *in situ* (epithelium)	Frequency
			methylation		100%
MSI					50%
3p21.3 – LOH, small telomeric deletions				contiguous deletions	80%
9p21-CDKN2 – LOH					70%
17p – LOH					
	MYC overexpression				60%
	?	**telomerase** dysregulation		**-telomerase** upregulation	80%
	?	**P53** + mutation			70%
	?		Aneuploidy		80%
		?	**FHIT**		40%
		cyclins +			
		bcl2>bax			
		8p21-23 – LOH			80%
			P16INK4		
			K-RAS mutation		20%
			Neoangiogenesis		40%
				5q21-APC-MCC-LOH	30%
				RB	

tic changes requires greater technical and financial demands, although some investigations are relatively easily performed by immune-histochemical methods.

Changes in the bronchial epithelium are very frequent, associated with various outdoor irritants, including infections, smoking etc. They may be solitary but mostly multiple, synchronous or metachronous, in different parts of the lung. Therefore, we can expect synchronous and metachronous lung tumours, which may appear in more than 10%, also based on our data. Together with the tumours of the upper respiratory system, which is exposed to similar or same noxious agents, the incidence of their multiplicity raises to 30%.

Goblet cell hyperplasia should be mentioned because of its role in etiopathogenesis of squamous metaplasia development. It is obvious that developed squamous metaplasia may derive either through basal cell hyperplasia or more frequently through goblet cell hyperplasia. In many cases, we have seen the goblet cell hyperplasia »undermined« with early squamous metaplasias with obvious intercellular bridges.

Dysplastic changes occur mostly in patients with diffuse or focal squamous metaplasia, and only in rare instances with diffuse epithelial hyperplasia. The follow-up of the patients with diffuse squamous metaplasia is therefore reasonable. Moreover, advanced dysplastic changes should be evaluated with great concern and care, especially if they are found in different dislocated regions of the lung. The use of proliferation markers or genetic molecular analysis is advisable to find out the high risk patients.

Very recently, there was a report about surviving, an inhibitor of apoptosis protein, which is absent in the normal epithelium,

non-neoplastic lung parenchyma adjacent to tumour, and in the low-grade atypical adenomatous hyperplasia. But it is expressed in the areas of squamous metaplasia and dysplasia as well as in the high grade atypical adenomatous hyperplasia adjacent to the tumour.[13] Very promising marker, if it will be proved true!

Dysplastic changes in the vicinity of foreign bodies, or caused by long-lasting mycotic infections probably exhibit lower proliferative capacity in comparison with dysplastic changes leading to invasive cancer or in the vicinity of developed lung cancer (Rott, personal unpublished observations). Therefore, in dubious cases, the use of markers of proliferative activity is recommended.

What is the reason for low incidence of intraepithelial carcinoma, carcinoma *in situ*? Does intraepithelial carcinoma rapidly change to invasive carcinoma? It is more likely that the undervaluation of dysplastic changes, which are at least in some cases de facto intraepithelial carcinoma, is the cause.

Diffuse idiopathic pulmonary neuroendocrine cells hyperplasia is not so rare as it was generally accepted. To reveal diffuse atypical hyperplasia of lung neuroendocrine cells, neuroendocrine markers should be used routinely in all resected specimens. In slices stained only with hematoxylin-eosin, without the use of neuroendocrine markers, this lesion may be practically overlooked in many cases. Probably we have missed some cases, because of inadequate sampling (only malignant tissue; or only a narrow rim of the pulmonary tissue, surrounding lung carcinoma, without included airways with respiratory epithelium). Therefore, we can conclude that diffuse idiopathic hyperplasia of pulmonary neuroendocrine cells appears at least in 6% of random selected surgical specimens. It is more common in women, especially in the fifth and sixth decades.[3]

Atypical adenomatous hyperplasia is a quite common lesion. It may appear in 4-35% in different lung cancer resection specimens.[3] Our surgical specimens were totally inadequate for the serious analysis of the incidence of atypical adenomatous hyperplasia. We have found it only in a few cases in the vicinity of adenocarcinoma. The localised lesions have usually less than 5 mm in diameter. Therefore, they are practically inaccessible for usual bronchial and transbronchial biopsies and standard x-ray investigations.

Aggressive small cell lung cancer has no known »ancestors« - precancerous lesions.

Although the main goal of all these investigations is detection of precancerous or early microinvasive cancer lesions, we should not exaggerate in overdiagnosing the actual lesions. It is much more reasonable to follow patients with suspicious lesions and to perform control biopsies.

Conclusions

Histology alone can find out early preinvasive epithelial lesions, which may proceed to invasive cancer types of non-small group. The additional molecular analysis with available immunohistology may be very helpful to find out the early signals of lung carcinogenesis. The patients with diffuse squamous metaplasia may develop reversible or even irreversible dysplastic changes. The follow-up of such patients and eventual rebiopsies are recommended. The neuroendocrine lesions require a routine demonstration of neuroendocrine component with adequate marker.

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