Lesions Preceding Squamous Cell Carcinoma of the Bronchus and Multicentricity of Canceration — Serial Slicing of Minute Lung Cancers Smaller Than 1 mm

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1993, **170** (1), 11-23 — A total of ten minute squamous cell carcinomas smaller
than 1 mm were found in surgical lung specimens from 108 patients who had
roentgenographically occult lung cancer. These minute lesions were detected by
submitting, in all the 108 specimens, the whole bronchial tree to 2-mm-thick
sequential transverse slicing which was then followed by microscopic examination
of each slice on an H-E stained section. When a focus of minute carcinoma was
found, the slice was further serially sectioned to study whether there were such
carcinoma-related lesions as dysplasia or other atypical changes of epithelia, and
when there were, the spatial relation of these with the carcinoma. It was demon-
strated that all the minute carcinomas were closely associated with either dysplasia
or what we call “basal cells with marked atypia”, cells with markedly enlarged
nuclei arranged in linear fashion on the basement membrane. The contiguity of
these changes with minute carcinoma strongly suggested that they are lesions
preceding overt carcinoma. Also, there were some minute foci of carcinoma,
which, though not involving the entire epithelial thickness, proved to have already
begun microinvasion. — roentgenographically occult lung cancer (ROLC);
minute carcinoma; dysplasia; basal cells with marked atypia; incomplete car-
cinoma

Despite an increase of the incidence of lung cancer, there has been no
satisfactory answer to its course of development. Theories concerning the devel-
opment of squamous cell carcinoma (SCC) of the bronchus (Melamed et al. 1977;

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Shimosato 1980; Melamed and Zaman 1982) do not necessarily offer conclusive evidence. The size of cancers they employed for their studies were much larger than those in our study, and it appears likely that a preceding lesion, even if present, had been destroyed by advancing carcinoma until the time of examination.

A mass screening for the detection of early lung cancer (the Miyagi Program), combining a miniature chest x-ray with sputum cytology, was initiated by our team in 1982 (Nakada et al. 1987). Since then, 108 patients with what we call roentgenographically occult lung cancer (ROLC) were detected by sputum cytology. By examining the resected specimens, tumors as small as less than 1 mm in transverse diameter were found. Such minute carcinomas were expected to be helpful in studying the steps preceding the development of SCC, since our working hypothesis is that the smaller the carcinoma size, the higher the probability of a preceding lesion remaining as it has been, not destroyed by carcinoma. Analysis of these minute carcinomas also gave us opportunities to visualize the multicentricity of canceration and the pathological background for the development of SCC in bronchi of heavy smokers. This report is a summary of the pathological characteristics.

MATERIALS AND METHODS

Between April 1982 and May 1989, 108 patients who were all male heavy smokers underwent lung resection for ROLC which was found by sputum cytology. Among them, 84 patients were detected in the Miyagi Program (Nakada et al. 1987) and the remaining 24 were referred to our outpatient clinic because of respiratory complaints. The locations of all the tumors of the above two groups were confirmed by repeated bronchoscopy prior to operation. All the resected specimens were fixed in 10% neutralized formalin. After fixation was completed, the bronchial trees were serially cut into 2-mm-thick sequential transverse slices from the margin of resection down to sub-subsegmental bronchi (Nagamoto et al. 1986). From the slices embedded in paraffin, microscopic sections 2.5 μ m thick were prepared from the proximal surface of each slice and were stained with hematoxylin and eosin. This produced an average of 37 blocks per specimen for segmentectomy (2 cases), 43 for lobectomy (77), 58 for bilobectomy (13), and 75 for pneumonectomy (16). In reviewing all these sections, 12 patients were found to have simultaneously multicentric cancers (10 double and 2 triple). Thus, a total of 122 ROLCs were found, which were all squamous cell type. Among the 122 SCCs, there was only one smaller than 1 mm in transverse diameter as measured by micrometer. The slice containing this cancer (Lesion A) was serially sectioned entirely at a thickness of 2.5 μ m for more detailed study.

In addition, a focus of atypical cells, when found in a slide, was also studied by serially sectioning the entire block. These serial sections were stained and examined every 10 sections: intermediate sections were stained and examined when necessary. This detailed study gave rise to the detection of a total of 9 minute carcinomas (Lesions B to J) which had a transverse diameter of less than 1 mm and were separate from the main tumor of each case. Thus, Lesions A to J served as the basic materials of this study (Table 1). They were subjected to a close histological examination of the cellular features in the carcinoma itself and its contiguous zones. A diagnosis of carcinoma in situ (CIS) was made according to the WHO classification (WHO 1981). Additional sections were stained with a silver impregnation method, PAS, Azan-Mallory and Elastica-Goldner stains mainly to confirm the continu-

TABLE I. *Summary of selected cases of minute carcinoma(s)*

Case Age (years)	Smoking index ^a	Main tumor			Minute carcinoma			Changes of adjoining epithelium
		Size (mm)	Location	Location	Lesion	Size (mm)	Microscopic appearance	
F.S. 51	20×30			B ^{8a}	A	0.5	In situ	D
M.K. 72	40×50	12	B ^{1+2a} +b					
		4	B ^{4a}	B ⁴	B	0.6	In situ	BCH
H.O. 59	40×25				C	0.3	Incomplete ^b	D
					D	0.8	In situ	NE & BCH
		26	B ⁶					
K.S. 67	25×40	12	B ⁹ /B ¹⁰ spur	B ^{8a}	E	0.8	Microinvasive	D
					F	0.5	Incomplete ^b	D
		2	B ^{2ai}	B ³	G	0.1	Microinvasive	D
T.Y. 61	40×40	6	B ³					
		2	B ^{2a} /b spur	B ³	H	0.2	In situ	BCMA & SMMA
S.O. 64	35×30	18	B ¹⁺² /B ³ spur	B ^{1+2aii}	I	0.1	Microinvasive	BCMA
					J	0.3	Microinvasive	BCMA

^aNumber of cigarettes smoked per day × Number of years smoked

^bIncomplete carcinoma = carcinomatous change is really seen involving only a part of the epithelial layer

BCH, basal cell hyperplasia; BCMA, basal cells with marked atypia; D, dysplasia; NE, normal epithelium; SMMA, squamous metaplasia with marked atypia.

ity of the basement membrane. When the continuity was apparently lost by carcinoma slightly invading down into the lamina propria, it was judged to be microinvasive as previously reported (Nagamoto et al. 1986). Involvement of bronchial glands was not counted into microinvasive carcinoma.

RESULTS

As shown in Table 1, epithelial changes in the zones contiguous to the 10 minute carcinomas were dysplasia, basal cells with marked atypia, squamous metaplasia with marked atypia, basal cell hyperplasia, and normal epithelium. The term "dysplasia" is a little confusing. According to the WHO classification (WHO 1981), dysplasia refers to a lesion in which the changes of cellular features are similar but less severe than those of CIS and typically do not involve the full thickness of epithelium. Spencer (1985) describes that it is a reversible state in which the number of epithelial cells is increased, the cells assume a spindle shape and are arranged perpendicularly to the surface, and in the superficial layer the cells may still remain ciliated. Tsuchiya et al. (1987) defined it as the stratified epithelium showing a certain degree of cellular and structural atypism, but not so severe as in carcinoma, with mitoses in the middle and upper layers. These descriptions mean that the definition of dysplasia has not yet been established in a form unanimously acceptable to many pathologists. Our criteria of dysplasia, basically according to the WHO classification (WHO 1981), was that it should be a highly cellular lesion showing cellular features similar to but less severe than those of CIS. As another cancer-related change, we defined "basal cells with marked atypia" as a lesion in which markedly atypical cells, larger than normal basal cells and with a large atypical nucleus, are arranged in linear fashion continuously along the basement membrane, but the atypism is not so marked as in CIS. Another expression "squamous metaplasia with marked atypia" was used basically according to the definition of Auerbach et al. (1957), but we confined the wording to stratified epithelia with less atypical cellular features than in dysplasia. "Basal cell hyperplasia" was used according to the definition of Auerbach et al. (1957).

Presentation of typical cases

Case M.K. In a slice of B⁴, taken from the immediate proximity of the main tumor, a CIS, 0.6 mm in diameter, was found growing in a strikingly bulky form (Lesion B). This lesion, as shown in Fig. 1a, was contiguous to basal cell hyperplasia on one side, and to a shallow erosion on the other, each with a distinct margin. Serial sectioning from Lesion B into the distal direction showed a duct of bronchial gland with epithelia entirely replaced by dysplasia. Further serial sectioning disclosed that this duct harbored an independent minute carcinoma 0.3 mm in diameter around the orifice (Lesion C) as indicated by arrowheads in Fig. 1b. This change did not involve the entire epithelial thickness, and a clear

Fig. 1. Case M.K.: Bars are 0.2 mm. H-E stain. (a) Lesion B; CIS (0.6 mm) contiguous to basal cell hyperplasia and shallow erosion. (b) Lesion C; A section closely distal to Lesion B disclosing a minute carcinoma (0.3 mm) at the orifice of a bronchial gland duct (arrowheads). This change does not involve the full thickness of epithelium with unclear border against the dysplastic zone. (c) Lesion D; A further distal section demonstrating CIS (0.8 mm) contiguous to normal ciliated epithelium and basal cell hyperplasia.

Fig. 2. Case H.O.: Bars are 0.2 mm. H-E stain. (a) A wide area showing dysplasia and a small group of atypical basal cells (arrows). (b) Lesion F; A much more distal section disclosing a minute carcinoma (0.5 mm) which shows a marked bulky outgrowth and disorderly arrangement of atypical cells (arrowheads) in the lower half of dysplastic zone. (c) A further distal section showing only dysplasia.

border was not definable against the dysplastic zone. In a more distal part of B4 there was dysplasia involving the same duct, but no carcinoma. In a further distal part (Fig. 1c) there was another CIS (Lesion D) of 0.8 mm in diameter,

independent of Lesion B, showing a marked thickening of epithelia and high cellularity; it proved to be contiguous partially to normal ciliated epithelium, but on the other side, it was surrounded partially by a zone of basal cell hyperplasia with a distinct margin. A further distal section disclosed only squamous metaplasia.

Case H.O. In a portion of B^{8a} separate from two main tumors, a wide area of dysplasia was detected (Fig. 2a). In its basal zone there was a small group of atypical basal cells. Serial sections starting from this disclosed that in the distal

Fig. 3. Case T.Y.: Bars are 0.2 mm. H-E stain. (a) A zone of dysplasia in the slight mucosal elevation. (b) Lesion H; A section distal to the above demonstrating CIS of 0.2 mm in diameter (arrowheads) sandwiched between squamous metaplasia with marked atypia and ciliated epithelium with markedly atypical basal cells extending along the basement membrane (arrows).

Fig. 4. Case S.O.: Bars are 0.2 mm. H-E stain. (a) A slice showing dysplasia with enlarged basal cells. (b) Lesion I; A serial section disclosing a minute carcinoma (0.1 mm) penetrating the basement membrane (arrowheads) at the orifice of bronchial gland duct. (c) Lesion J; A further distal section revealing another carcinoma (0.3 mm) invading the lamina propria (arrowheads) without involving the full epithelial layer. It is surrounded by dysplastic area including basal cells with marked atypia arranged in linear fashion (arrows) along the basement membrane.

vicinity, there was a small focus of large atypical cells extending from the basal to the superficial zone. This proved to form a carcinoma 0.8 mm in diameter (Lesion E) with high cellularity, growing markedly downward and continuing to dysplastic zones in the surroundings. In a much more distal section there was another minute carcinoma of 0.5 mm in diameter (Lesion F) showing a marked bulky outgrowth and disorderly arrangement of atypical cells in the lower half of epithelium (Fig. 2b); this was separate from Lesion E, but was surrounded by zones of dysplasia with obscure margins. In sections distal to Lesion F, there was only dysplasia (Fig. 2c). Thus the two carcinomas turned out to be buried in a common focus of dysplasia which occupied an area of 4×4 mm.

Case T.Y. A zone of dysplasia was found as a slight mucosal elevation (Fig. 3a) continuous to the main tumor in B³. Serial sections from this level disclosed a minute CIS (Lesion H) of 0.2 mm in diameter (arrowheads in Fig. 3b) which was sandwiched between an area of squamous metaplasia with marked atypia and that of ciliated epithelia with markedly atypical basal cells extending along the basement membrane. Bilaterally, margins of this CIS were ambiguous. Serial sections distal to Lesion H demonstrated only a small focus of dysplasia like that shown in Fig. 3a.

Case S.O. In a slice directly distal to the main tumor arising in B¹⁺²aii, there was an area of dysplasia with enlarged basal cells (Fig. 4a). In serial sections that followed, there was a minute carcinoma of 0.1 mm in diameter (Lesion I). As shown in Fig. 4b, this carcinoma already penetrated the basement membrane around the orifice of bronchial gland duct, though it had not involved the full layer of epithelium. A slightly distal section showed another carcinoma (Lesion J) separate from Lesion I, which, as shown in Fig. 4c, was apparently invading the lamina propria, again without involving the full thickness of epithelium. This was surrounded by zones of dysplasia having a markedly atypical basal cells along the basement membrane. Sections distal to this demonstrated only dysplasia. The size of the dysplastic area containing Lesions I and J was 2×2 mm.

DISCUSSION

Our discussion on lesions preceding squamous cell carcinoma (SCC) is based on histological findings of minute carcinomas less than 1 mm in diameter. We are of the opinion that analysis of such minute carcinomas is essential in studies of carcinogenesis since a preceding lesion often remains untouched by carcinoma. Wakimoto et al. (1982) described that 76.1% of squamous metaplasias were less than 3 mm in width. The extent of carcinomas in situ (CISs) reported by Melamed et al. (1977) ranged from 5×5 to 30×30 mm. Those by Shimamoto (1980) were also small, the smallest being 2 mm in diameter. Both authors stated that SCC may arise from epithelia appearing normal.

Also in the present investigation, there was a tumor (Lesion D) which was contiguous to normal ciliated epithelia, a finding that may lend support to their

contention. However, Lesions A and C were contiguous to dysplasia with indistinct borders, and Lesions E and F were buried in a focus of dysplasia. These findings strongly suggest that in a majority of bronchial SCCs, dysplasia precedes overt carcinoma. Lesions I and J were also surrounded by dysplastic areas where basal cells were particularly atypical. It appears from these findings that "basal cells with marked atypia" are a lesion which could develop into dysplasia. In Lesion H, these gave rise to formation of an area sufficiently atypical as to allow one to classify as carcinoma. As shown in Figs. 3b and 4b, these atypical cells are much larger than normal basal cells and have a large atypical nucleus. They are lined continuously along the basement membrane as one or two-cell layers, while the individual cells present marked polymorphism. These atypical cells, confined in the basal layer, are quite unlikely to correspond to laterally invading carcinoma. They also appear different from what is called basal cell atypia in the report of Melamed and Zaman (1982), which they regarded as one of the recognizable preceding lesions in the development of SCC. Apparently the basal cell atypia stressed by the above authors differs from the finding we described above, the large atypical cells arranged in linear fashion on the basement membrane. Probably "basal cell atypia" in their studies were similar to the change shown in Fig. 2a. The present observation makes us imagine that in the basal cell atypia we defined, dysplasia, or, carcinoma can directly develop. But such basal cell atypia was detected in only one case (Case H.O.).

On the other hand, basal cell hyperplasia and squamous metaplasia are regarded as one of the steps in the course of carcinoma development. As Melamed et al. (1977) states in their report, these two types of changes are regarded as reversible, not necessarily preceding overt SCC. However, if they are associated with marked atypia, they could be qualified as a lesion closely related with carcinoma as shown in Fig. 3b where squamous metaplasia with marked atypia was noticed to be contiguous to carcinoma. Lee et al. (1989) demonstrated dynamic changes of *c-myc* oncogene expression by in situ hybridization, in a sequential cascade from squamous metaplasia to dysplasia to SCC of the bronchial epithelium. Fisseler-Eckhoff et al. (1990) reported by immunohistochemistry such characteristic changes of extracellular matrix components as loss of fibronectin, collagen type III and laminin in the basement membrane to correlate with an increasing degree of epithelial dysplasia. Both reports regarded dysplasia as a precancerous lesion just preceding overt carcinoma, but they did not refer precisely to the degree of atypia in squamous metaplasia. As indicated by these authors, it seems reasonable to think that the squamous metaplasia potentially can develop into dysplasia as the degree of atypism increases.

Since there is no staining specific to carcinoma cells, histological identification of extremely small carcinoma is difficult. So long as complying with the criteria of WHO classification (1981) where CIS was defined as a lesion involving the full thickness of epithelium, even an undoubtedly carcinomatous

Fig. 5. A scheme of development of squamous cell carcinoma of the bronchus in heavy smokers. The solid lines show the steps deduced from our data and the dotted lines the recognizable steps.

change cannot be diagnosed as such if it does not involve the full epithelial thickness. In reality, however, there does exist a carcinoma smaller than 1 mm, which involves only a part of the epithelial layer. It seems appropriate to call such a lesion “incomplete carcinoma”. Its cellular features are apparently different from those of dysplasia (Spencer 1985; Tsuchiya et al. 1987), but look carcinomatous, as shown in Figs. 1b and 2b. We believe that “incomplete carcinoma” already behaves as a malignant lesion. The finding in Lesions G, I and J where the atypical cells were beginning microinvasive growth without involving the entire thickness of epithelium strongly suggests that a minute carcinoma could invade the basement membrane even before it has involved the full thickness of epithelium.

Based on the above, we summarize the developmental steps of SCC as in Fig. 5. It clearly indicates that in the histogenesis of SCC, dysplasia and basal cells with marked atypia are the main conditions preceding overt SCC.

Roentgenographically occult lung cancer (ROLC) constitutes a very small group in the whole population of lung cancer (Martini and Melamed 1980). However, it is interesting that the rate of simultaneously multifocal primary carcinomas of the lung is very high among patients who had a history of heavy smoking (Martini and Melamed 1980; Eggleston et al. 1982; Woolner et al. 1984). It is surprising that the rate of detection of a second primary carcinoma is almost 10 times higher than the incidence rate of lung cancer among heavy smokers (Woolner et al. 1984). A possible explanation may be that ROLC, arising in the bronchial mucosa of smokers exposed to tobacco over a long period, is essentially multifocal in origin, since smoking induces simultaneous metaplastic, hyperplastic and/or dysplastic changes of mucosa. The statement of Auerbach et al. (1961) is valid: tobacco causes epithelial changes with atypia, where the number of atypical cells greatly increases with the increased amount of cigarette smoking. The epithelial atypia occurs at different sites in the bronchial tree, which probably

develops into dysplasia or markedly atypical basal cells arranged in linear fashion. Our study reveals that the patients of our series had multiple carcinomas and suggests that both dysplasia and basal cells with marked atypia are a lesion preceding carcinoma. The presence of preceding lesions have been confirmed in carcinomas of different portions, suggesting the possibility of essentially multifocal carcinogenesis progressing over a long period. However, not all the preceding lesions develop into a carcinoma. Either at the same time or over a period of many years, only a small portion of them are supposed to transform into carcinomas which usually appear as multiple primary cancers (Saccomanno et al. 1974). In cases of multiple tumors, not all foci of carcinomas could be detected at the same time, probably because the growth rate might be different from one tumor to another (Nagamoto et al. 1989). One of two carcinomas, when arising much later than the other, may tend to be much smaller. Carcinomas smaller than a certain limit cannot necessarily be detected by bronchoscopy and brushing cytology. On the other hand, as shown in our study, more than one carcinoma can occur in one preceding lesion. It may also be possible, however, that they grow and merge into a single tumor before they are detected. Therefore the possibility is high that multiple cancers are judged to be solitary. This may be synonymous with saying that, in the very early stage of lung cancer, multiple tumors may be more frequent than they appear.

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