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CASE REPORT

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Multifocal micronodular pneumocyte hyperplasia in a postmenopausal woman with tuberous sclerosis

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Abstract We report a peculiar case of multifocal micronodular pneumocyte hyperplasia (MMPH) without association of pulmonary lymphangioleiomyomatosis (LAM) in a 56-year-old postmenopausal woman with tuberous sclerosis. This case is surmised to be a *forme fruste* of tuberous sclerosis. Computed tomography demonstrated multiple micronodules, measuring up to 5 mm in size, present in the bilateral lung fields, but no cystic changes. A proliferation of pleomorphic type-II pneumocytes lining the thickened alveolar septa in an adenomatoid pattern, with an associated increase in alveolar macrophages, was observed without typical nuclear atypia. In fully developed lesions, the ingrowth of more proliferating type-II pneumocytes into the thickened alveolar septa and macrophages filling the alveolar lumens were characteristic findings. Proliferation of immature smooth muscle cells suggesting LAM was not observed. Positive immunohistochemical stains for cytokeratin, epithelial membrane antigen, and surfactant apoproteins A and B, and negative staining for HMB45, alpha-1 smooth muscle actin, desmin, and carcinoembryonic antigen confirmed the characteristics of alveolar lining cells in each MMPH lesion. MMPH associated with tuberous sclerosis in the postmenopausal woman appears to be similar to that described in premenopausal women. The present case is familial rather than sporadic and suggests no relationship between the development of MMPH and the underlying hormonal state.

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Introduction

Tuberous sclerosis is a rare autosomal dominant disorder characterized by cutaneous lesions (angiofibroma and subungual fibroma), epilepsy, and mental retardation. As pulmonary involvement, lymphangioleiomyomatosis (LAM) is found in 1% of cases [3], while multifocal micronodular pneumocyte hyperplasia (MMPH) is extremely rare [11]. The majority of patients with MMPH in tuberous sclerosis are women of premenopausal age associated with LAM. In this report, we describe a postmenopausal woman with tuberous sclerosis and pulmonary involvement manifested by MMPH without LAM.

Case report

A 56-year-old woman had been diagnosed 2 years previously as having tuberous sclerosis because of angiofibroma of the face and the left thigh, and subungual fibroma. Both of her two married daughters, aged 33 years and 27 years, had also been diagnosed as having tuberous sclerosis. The patient was referred in August 1997 for systemic screening examination for tuberous sclerosis. Her menopause occurred at the age of 51 years. She had no smoking habit or history of respiratory symptoms and had remained free of respiratory symptoms. Although she had neither mental retardation nor history of epileptic seizure, she had been administered an anti-epileptic drug for tuberous sclerosis during these 20 months but had never received sex-hormonal therapy. Neurologic examinations revealed no abnormalities. Computed tomography (CT) and magnetic resonance imaging (MRI) of the head revealed subependymal calcifications and multiple lacunae in the bilateral deep white cortices. Echogram and MRI of the neck suggested multiple small cysts (follicular adenoma) in the bilateral lobes of the thyroid. Echocardiogram revealed mild aortic regurgitation. Urinalysis and screening abdominal echogram revealed normal findings.

Lung function tests were normal. However, the blood gas analysis revealed hypercapnia (PCO_2 48 mmHg) and hypoxemia (PO_2 76 mmHg). Chest X-ray showed almost normal findings with

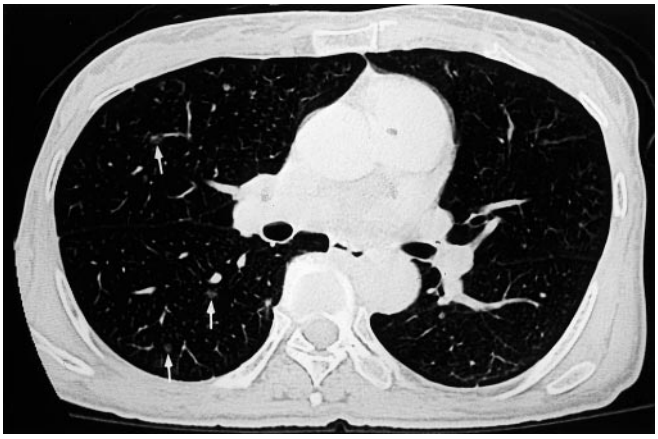

Fig. 1 Chest computed tomography (CT) shows multiple micronodular shadows (*arrows*) smaller than 5 mm in diameter in the bilateral lung fields. However, cystic changes suggesting lymphangioleiomyomatosis (LAM) are absent

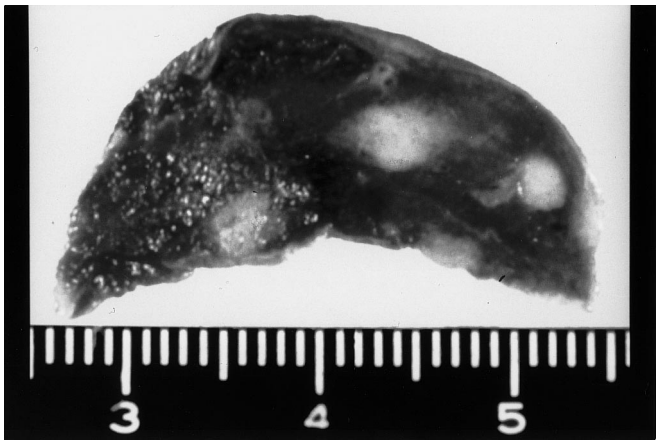

Fig. 2 The cut surface of the removed specimens shows multiple white firm nodules of varying size ranging from 1 mm to 5 mm in diameter, scattered haphazardly in the lung parenchyma. Cystic and emphysema-like lesions are not observed

faintly recognizable bilateral small nodular opacities. Bullous changes, interstitial fibrosis, increased lung volume, or pleural effusions were not recognized. Chest CT revealed multiple micronodular shadows smaller than 5 mm in diameter in the bilateral lung fields throughout, without cystic changes suggestive of LAM (Fig. 1). To obtain a histopathologic diagnosis, specimens were resected from the right lung using a thoracoscopic procedure. She is doing well 20 months postoperatively.

Materials and methods

The resected lung tissues were fixed in 10% neutral formaldehyde solution and embedded in paraffin using conventional techniques. All the sections were stained with hematoxylin and eosin and elastica van Gieson stain. Immunohistochemical examination was performed using the labeled streptavidin–biotin method, using commercially available antibodies, each of which was obtained from Dako (Denmark). As for surfactant apoproteins A and B used in this study, the antibodies were produced by means of monoclonal technology using purified surfactant apoproteins of alveolar lavage from a patient with alveolar proteinosis as antigens [13]. Endogenous peroxidase was blocked by hydrogen peroxide, but no protease pretreatment was necessary. The sections were stained immunohistochemically with antibodies against cytokeratin, epithelial membrane antigen (EMA), surfactant apoproteins A and B, carcinoembryonic antigen (CEA), alpha-1 smooth muscle actin, desmin, and HMB45.

Results

Thoracoscopy showed multiple small white nodules up to 5 mm in diameter, which were scattered in the subpleural lung parenchyma in each lobe of the right lung. Localization of pulmonary lesions was dominant in the lower lobe. The lung surface showed no cystic lesions suggestive of LAM. Resected specimens were obtained from three lobes (segments 2, 4, and 6). The cut surface of the removed specimens showed multiple white firm nodules of varying sizes ranging from 1 mm to 5 mm in diameter, scattered haphazardly in the lung parenchyma (Fig. 2).

Histopathologically, these multiple nodules were relatively well demarcated and consisted of proliferation of type-II pneumocytes. The stroma of these nodules showed fibrous thickening of the alveolar septa with elastosis on an elastic tissue stain. Proliferative pneumocytes and moderate fibrous and hyalinous thickening of alveolar septa were observed in the diseased areas as well as papillary architecture. The type-II pneumocytes which varied in size and were pleomorphic in shape, occasionally showed somewhat flattened cells with hyperchromatic nuclei that resembled transformed cuboidal cells with chromatin aggregations and prominent nucleoli. However, they lacked marked nuclear atypia or mitotic figures. A few multinucleated cells and intranuclear eosinophilic inclusions were noted. Mild infiltration of lymphocytes and eosinophils in the thickened alveolar septa and aggregations of macrophages in the alveolar lumens were also observed (Fig. 3). Proliferation of immature smooth muscle cells suggesting LAM was not observed, including in the areas of thickened alveolar septae or around pulmonary arteries and bronchioles. Emphysema-like lesions as seen in cases of LAM were also not observed. Neither necrosis nor vascular invasion was noted. Lepidic spread away from the nodules or microscopic cystic changes was absent. Dilated lymphatic vessels in the lung parenchyma were observed.

In the immunohistochemical investigations, all proliferating alveolar epithelial cells were intensely stained by cytokeratin and EMA antibodies. In addition, these cells were positive for monoclonal antibodies for surfactant apoproteins A and B. Antibodies directed against CEA gave completely negative results. Proliferating epithelial cells remained unstained with desmin, alpha-1 smooth muscle actin, or HMB45. The microvessels within the thickened alveolar septa were positive for alpha-1 smooth muscle actin, rarely positive for desmin, but negative for HMB45. With HMB45 antibodies, all lung parenchymal cells were negative.

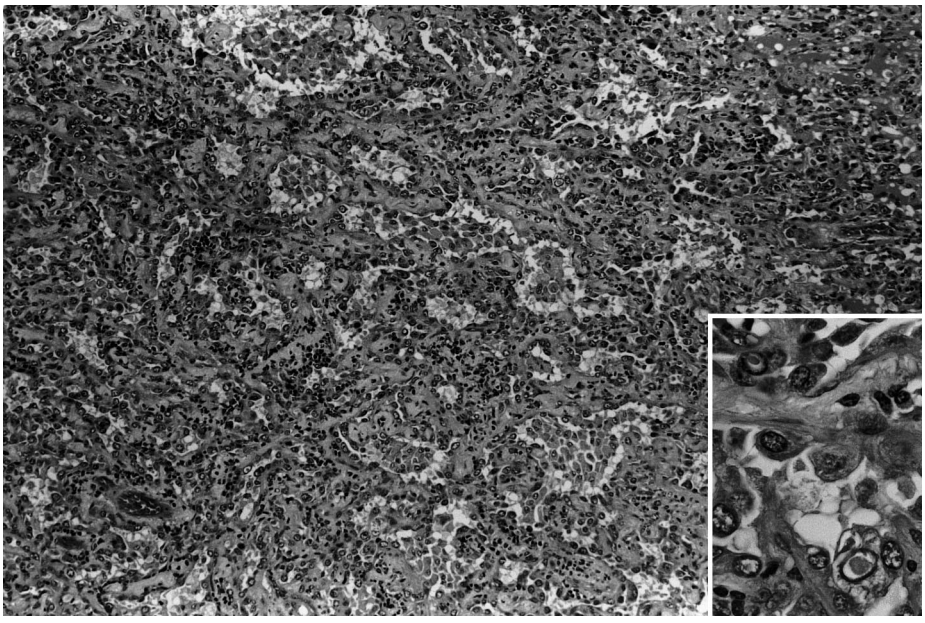

Fig. 3 Histopathological findings of the nodule consist of proliferation of type-II pneumocytes with fibrous thickening of alveolar septa. The type-II pneumocytes are pleomorphic in shape with prominent nucleoli. The ingrowth of proliferating epithelial cells into the thickened and distorted alveolar septa is characteristic. Infiltration of lymphocytes and eosinophils in the thickened alveolar septa and aggregations of macrophages in the alveolar lumens are observed. Higher magnification of the central area showing intranuclear eosinophilic inclusions (*inset*). (Hematoxylin and eosin, original magnification ×100. Inset ×400)

Discussion

LAM is more frequently associated with tuberous sclerosis as a pulmonary involvement of tuberous sclerosis than with MMPH or clear cell "sugar" tumor [8]. MMPH in patients with tuberous sclerosis is also associated with LAM [5, 7, 8, 9, 11, 14, 15, 16], but is rarely observed without LAM [11, 16]. Although MMPH may be regarded as a distinctive lesion developing in the lung of patients with tuberous sclerosis, it is also observed rarely among patients without tuberous sclerosis [3, 16, 17]. LAM in postmenopausal women who had never received oophorectomy or hormonal therapy has been reported very rarely [2, 10]. However, there have been no reports of MMPH in postmenopausal women such as our case. The present case is the oldest female patient with MMPH to our knowledge.

Histopathologically, there was no evidence of LAM, but multiple nodules consisting of proliferation of type-II pneumocytes were evident in our case. They showed adenomatous pneumocyte hyperplasia in parts, but the general aspect of MMPH seems to demonstrate the ingrowth of proliferating epithelial cells into the alveolar septa. Intranuclear eosinophilic inclusions [14, 16] and inflammatory infiltrates [16] are noticed, as previously mentioned. In early lesions, there was a marked proliferation of epithelial cells on the surface of the alveoli and only a little penetration of cells into the septa; i.e., (multiple) adenomatoid proliferation [7, 15] or adenoma-like pattern [14] as previously described. However, in fully developed lesions, more epithelial cells were found within the alveolar septa. There were clusters of macrophages in the alveolar lumen [16]. The present case showed variable manifestations of pneumocyte hyperplasia that demonstrated lining alveolar septa and nodular aggregation within the alveolar septa. As for the origin of the proliferating epithelial cells, it is controversial whether only type-II pneumocytes [9, 16, 18] or also Clara cells are present in addition to type-II pneumocytes [14]. Intra-alveolar macrophage aggregation and dilated lymphatic vessels were demonstrated microscopically in our case, possibly due to secondary manifestations of occlusion or stenosis of proximal lymphatic vessels or airways by the epithelial proliferation, as Popper [18] described in a case which showed dilated alveoli and lymphatic vessels with no evidence of LAM histologically. Thus, MMPH demonstrates a morphological spectrum [3, 18] in each nodule according to the grade of pneumocyte hyperplasia, in addition to secondary manifestations such as intra-alveolar macrophage aggregation, dilated lymphatic vessels, and cystic dilated alveoli.

For the differential diagnosis, adenomatous hyperplasia (AH), atypical adenomatous hyperplasia (AAH), so-called sclerosing hemangioma, bronchioloalveolar carcinoma, or carcinoid tumorlet should be included. In our case, the diagnosis of MMPH was confirmed by the presence of solid condensed areas and of poor alveolar structures distinguished from AH or AAH. AAH, showing occasional immunoreactivity for CEA and p53 [12, 19], consists of epithelial cells with nuclear atypia and high nuclear-to-cytoplasmic ratio, and shows alveolar structures with fewer intra-alveolar macrophages than MMPH [16]. So-called sclerosing hemangioma often presents a solitary mass lesion [14, 20, 21] and shows a mixed pattern with a well-demarcated circumferential margin. It is also distinguished from bronchioloalveolar carcinoma or adenocarcinoma by the lack of frank nuclear atypia and mitoses. (Multicentric) Bronchioloalveolar carcinoma has a feature of prominent peripheral lepidic spread [16] and does not contain solid condensed areas compared with MMPH. AAH and bronchioloalveolar carcinoma may show a mucinous or non-mucinous pat-

tern and the alveolar walls may be intact and not fibrotic unlike MMPH. Chemodectoma-like bodies consist of micronodules which have a perivenous location and no capillary network [14, 18], and show immunoreactivity for EMA but not for cytokeratin [5, 9, 14, 18]. Carcinoid tumorlets, which consist of small proliferations of neuroendocrine cells, may be found in a fibrous stroma and show peribronchiolar or intra-alveolar growth [1, 6]. Neuroendocrine cell hyperplasia consists of numerous neuroendocrine cells or bodies that proliferate within the airway epithelium [1, 6].

On immunohistochemical analysis, the epithelial cells strongly expressed cytokeratin, EMA, and surfactant apoproteins A and B, but not CEA, desmin or HMB45. Previous authors' descriptions [9, 14, 18] were similar to ours, but Muir [16] described less prominent but positive staining for CEA. MMPH that shows no invasion into blood or lymphatic vessels [14, 18] or no immunohistochemical reactivity for p53 or CEA [14] is considered to possess no malignant potential. HMB45 is recognized as a marker for LAM immunohistochemically distinct from other pulmonary smooth muscle proliferation [4]. Hitherto, all authors have described no immunoreactivity for HMB45, in addition to negative results for estrogen and progesterone receptors in MMPH [5, 8, 9, 14] unlike in LAM [5, 14]. In the cases with combined LAM and MMPH, the characteristic manifestation of LAM was not identified as part of MMPH, and HMB45-positive cells of LAM ended at the edge of the MMPH lesion [16]. Negative staining for HMB45 in MMPH lesions indicates that the pathogenesis of MMPH is probably separate from the smooth muscle cell proliferation of LAM [8, 9, 14, 16].

MMPH associated with tuberous sclerosis in postmenopausal woman has not been reported to our knowledge, but it appears to be similar to that previously described in premenopausal women and in a man [17]. The present case is familial rather than sporadic, and is not associated with LAM. Although rare, MMPH has been observed in males [11, 16, 17]. The MMPH in the present case was diagnosed in the postmenopausal woman and suggests that MMPH developed without relationship to the patient's estrogen level.

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