GENERAL THORACIC SURGERY

DIFFERENTIAL DIAGNOSIS OF THYMIC CARCINOMA AND LUNG CARCINOMA WITH THE USE OF ANTIBODIES TO CYTOKERATINS

There are few specific pathologic findings that can be relied on to distinguish primary thymic carcinomas from lung carcinomas with mediastinal extension or showing metastasis to the anterior mediastinum. The immunohistochemical reactivity on frozen sections of thymic carcinomas and lung carcinomas, which are histologically similar to each other, was examined with the use of monoclonal antibodies to cytokeratins 7 and 13. Among keratinizing squamous cell carcinomas, all thymic carcinomas reacted with antibody specific for cytokeratin 7 (9/9, 100%), whereas no staining reaction was seen in lung carcinomas (0/5, 0%) ($p < 0.01$). This finding can be used as a diagnostic aid in primary thymic keratinizing squamous cell carcinomas to expedite treatment and prognosis. Cytokeratin 7 and cytokeratin 13 monoclonal antibodies reacted with almost all cases of thymic carcinoma. Applications of monoclonal antibodies specific for certain cytokeratins, especially 7 and 13, may be helpful in the diagnosis of other subtypes of thymic carcinomas. (J THORAC CARDIOVASC SURG 1995; 110:1670-5)

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Because of their relative rarity and close histologic similarity to lung carcinomas, primary thymic carcinomas might be misdiagnosed as lung carcinomas involving the mediastinum. Among the wide variety of morphologic appearances, squamous cell carcinoma (SCC) represents a common type of thymic carcinoma. Therefore it is important to differentiate thymic SCC from lung SCC with mediastinal extension, because the former appears to have a better prognosis in some instances, when properly treated.^{1,2}

Cytokeratins are a complex group of substances

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comprising at least 19 different polypeptides, which range in molecular weight from 49,000 to 68,000 daltons. There are different combinations of cytokeratins in epithelial cells, depending on the cell type, and epithelium-derived tumors maintain the expression of some of the cytokeratin polypeptides typical of the specific nontransformed cells.^{3,4} We previously reported that human thymic epithelial cells could be subdivided in terms of their expression of cytokeratin.⁵ A given thymic carcinoma can therefore be distinguished from a lung carcinoma, if the combination of cytokeratin polypeptides in thymic tissue is different from that in bronchial tissue. The present study was undertaken to determine whether some cytokeratins not expressed in lung carcinomas may, in fact, be present in thymic carcinomas.

Material and methods

Tissues. Between 1984 and 1993, samples from 98 cases of thymic epithelial tumors were collected from various hospitals in Japan. Among the samples, 17 were identified as thymic carcinomas histologically. The cases in the present study were those of cytologically malignant epithelial tumors located in the anterior mediastinum. This criterion for selection included the absence of a primary tumor at sites other than the anterior mediastinum at the

time of presentation.^{2,6} In addition, five biopsy samples of normal thymus tissue were collected from children at the time of operation for funnel chest after parental consent was obtained.

Fresh specimens of tumors and normal thymus tissue were snap-frozen in Optimum Cutting Temperature medium (Bayer Corp., Elkhart, Ind.) and stored at -75°C for immunoperoxidase staining. Portions of the specimens fixed in 10% formalin were embedded in paraffin for routine histologic studies.

Mouse monoclonal antibodies. Two mouse monoclonal antibodies were used in this study. The first, RCK105 (immunoglobulin G1),⁷ in immunoblotting reacts only with single cytokeratin polypeptide cytokeratin 7 and stains a subgroup of glandular epithelia and their tumors, next to transitional bladder epithelium and bladder carcinomas. The second, 2D7 (immunoglobulin G2b),⁸ recognizes only cytokeratin 13 in immunoblotting studies and reacts with noncornifying squamous epithelia. Both were purchased from ICN Corporation (Lisle, Ind.). Neither RCK105 nor 2D7 is applicable for formaldehyde-fixed paraffin-embedded tissues.

Staining procedure. Serial sections, 4 μm thick, were fixed in acetone at 4°C for 10 minutes, air dried, washed in phosphate-buffered saline solution (pH 7.2), treated with 5 mmol/L periodic acid for 10 minutes at room temperature to remove endogenous peroxidase activity,⁹ and reacted with primary mouse monoclonal antibodies for 60 minutes at room temperature. The sections were stained by the streptavidin-biotin-peroxidase complex method with the Histofine kit (Nichirei, Tokyo, Japan). RCK105 and 2D7 were applied at a dilution of 1:10. The sections reacted in a solution consisting of 20 mg of 3,3'-diaminobenzidine-4HCl in 100 ml of 0.005 mol/L Tris HCl buffer (pH 7.6) containing hydrogen peroxide 0.005%; this reaction was complete in 10 minutes. Next, the sections were washed in phosphate-buffered saline solution, counterstained with methyl green, dehydrated in a graded alcohol series and xylene, and mounted with synthetic resin. For negative control specimens, phosphate-buffered saline solution and normal mouse serum (1:10 dilution) were used instead of the primary mouse monoclonal antibodies.

Results

Thymus. The localization of epithelium expressing cytokeratin 7 and cytokeratin 13 is shown in Table I. The monoclonal antibody 2D7, which is specific for cytokeratin 13, labeled some subcapsular-cortical epithelial subsets and medullary epithelium except for a minor cell subset localized near the cortex. The monoclonal antibody RCK105, which is specific for cytokeratin 7, labeled a somewhat smaller number of subcapsular-cortical and medullary epithelial cells than 2D7 (Fig. 1).

Tumors. Seventeen thymic carcinomas were classified according to the published criteria² into nine well-differentiated (keratinizing) SCCs, five lymphoepithelioma-like carcinomas, two undifferentiated

Table I. Localization of cytokeratins 7 and 13 in epithelial cells in normal thymus

No. of cytokeratins*	Cortex		Medulla	
	Subcapsular	Intracortical	Periphery	Inner
CK7	—~±	—	—	±
CK13	±	—	—	+

CK, cytokeratin; —~±, rare cells; —, negative; ±, multiple positive cells; +, majority of positive cells.

*Moll's classification.

carcinomas, and 1 mucoepidermoid carcinoma. Morphologically, well-differentiated (keratinizing) SCCs by published criteria² appear to correspond to well-differentiated to moderately differentiated pulmonary SCCs of lung origin. Five cases of well-differentiated to moderately differentiated pulmonary SCCs were also studied. For the sake of convenience, we here use the term *keratinizing SCCs* for both thymic well-differentiated (keratinizing) SCCs and well-differentiated to moderately differentiated pulmonary SCCs.

Cytokeratin 7 and cytokeratin 13 expression in thymic carcinomas and lung carcinomas is summarized in Table II. Among keratinizing SCCs, all thymic carcinomas reacted with RCK105 (9/9, 100%), whereas no staining reaction was seen in lung carcinomas ($p < 0.01$) (Fig. 2). The monoclonal antibody 2D7 reacted with all cases of thymic lymphoepithelioma-like carcinoma (Fig. 3). Both RCK105 and 2D7 reacted with undifferentiated carcinomas and mucoepidermoid carcinoma of thymic origin.

Discussion

In any case in which thymic carcinoma is considered to be the diagnosis, exhaustive clinical investigation to rule out a primary extrathymic neoplasm is required, because there are few specific pathologic findings that can be relied on to distinguish primary thymic carcinomas from the metastatic neoplasms that are so histologically similar.⁶ However, in some instances, it might be impossible to differentiate thymic carcinoma from lung carcinoma involving the mediastinum or showing metastasis to the anterior mediastinum.

Thymic carcinomas can be subclassified into categories including well-differentiated (keratinizing) SCC, lymphoepithelioma-like carcinoma, small cell carcinoma, sarcomatoid carcinoma, clear cell carcinoma, mucoepidermoid carcinoma, basaloid carcinoma, and undifferentiated carcinoma. Of these,

Fig. 1. Double immunolabeling of normal human thymus tissue with RCK105 and 2D7 (mirror sections). **A**, RCK105 (original magnification $\times 20$). **B**, 2D7 (original magnification $\times 20$). RCK105 labeled somewhat smaller number of medullary epithelial cells than 2D7.

well-differentiated (keratinizing) SCC and lymphoepithelioma-like carcinoma represent the relatively frequent types.² Morphologically, thymic well-differentiated (keratinizing) SCCs appear to correspond to pulmonary well-differentiated to moderately differentiated SCCs, that is, keratinizing SCCs, and lymphoepithelioma-like carcinoma is a unique morphotype that is only rarely seen in primary tumors of the lung.¹⁰ Therefore it is desirable to

differentiate keratinizing SCCs of thymic origin from those of lung origin with mediastinal extension or showing metastasis to the anterior mediastinum. The former appears to have a better prognosis, if properly treated.^{1,2}

With the use of monoclonal antibodies specific for a single cytokeratin, we earlier reported that thymic epithelial cells could be subdivided in terms of their cytokeratin expression and found that discrete med-

Fig. 2. Hematoxylin and eosin–stained sections of thymic keratinizing SCC (A) and pulmonary keratinizing SCC (B) (original magnification $\times 80$). Cytokeratin 7 antibody RCK105 shows completely positive reaction in thymic keratinizing SCC (C), but does not react with pulmonary keratinizing SCC (D) (original magnification $\times 80$).

Table II. Expression of cytokeratins in thymic carcinomas and lung carcinomas

	Origin	CK7	CK13
Keratinizing SCCs	Thymus	9/9 (100%)	8/9 (88.9%)
	Lung	0/5 (0%)	4/5 (80%)
Lymphoepithelioma-like carcinoma	Thymus	3/5 (60%)	5/5 (100%)
Undifferentiated carcinomas	Thymus	2/2 (100%)	2/2 (100%)
Mucoepidermoid carcinoma	Thymus	1/1 (100%)	1/1 (100%)

* $p < 0.01$.

ullary epithelial subsets located near the Hassall's corpuscles expressed cytokeratin 18 and the majority of the epithelial cells of thymic carcinoma expressed cytokeratin 18.⁵ Immunohistochemical analysis by Broers and colleagues¹¹ indicated that the presence of cytokeratin 7, normally occurring in lung adenocarcinomas, varied in lung SCC from no positive cells in well-differentiated SCCs to a positive reaction in all tumor cells for poorly differentiated SCCs. They suggested the presence

of varying degrees of adenocarcinoma differentiation within SCCs, whereas in lung SCCs histologic squamous cell differentiation has been found in parallel with the presence of cytokeratin 13.¹¹ Cytokeratin 13 antibody thus reacts with many more cases and many more cells of keratinizing SCC as compared with its reaction in poorly differentiated SCC. All keratinizing SCCs of thymic origin reacted with RCK105 (cytokeratin 7) in the present study, whereas no staining reaction

Fig. 3. A, Hematoxylin and eosin-stained section of lymphoepithelioma-like thymic carcinoma (original magnification $\times 80$). B, Large areas of this tumor are stained with cytokeratin 13 antibody 2D7 (original magnification $\times 80$).

was seen in those of lung origin. This result is highly indicative of the possibility of differential diagnosis between thymic keratinizing SCCs and lung keratinizing SCCs.

Broers and colleagues¹¹ indicated the possibility of limited focal reaction with cytokeratin 7 antibody RCK105 in lung keratinizing SCCs because of tumor heterogeneity. However, thymic carcinomas, which probably originate in thymic medullary epi-

thelium,⁵ would ordinarily tend to exhibit much larger quantities of cytokeratin 7 positive cells in cases of keratinizing SCC than would be seen in lung SCCs.

In conclusion, we believe that establishing the value of immunolabeling for cytokeratin 7 in the diagnosis of primary thymic keratinizing SCCs may have a decidedly favorable impact on treatment and prognosis. Because both RCK105 (cytokeratin 7)

and 2D7 (cytokeratin 13) reacted in almost all cases of thymic carcinoma, the possibility exists that other subtypes of thymic carcinomas comparable to those seen in lung, nasopharynx (lymphoepithelioma), kidney, and salivary gland⁶ may be differentiated from metastasis to the anterior mediastinum from a tumor in those sites by the use of monoclonal antibodies specific for certain cytokeratins especially cytokeratins 7 and 13.

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