The Biologic Spectrum of Thymic Epithelial Neoplasms: Current Status and Future Prospects

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Thymoma, thymic carcinoma, Masaoka stage, targeted therapy, mediastinal neoplasms, EGFR, Kit (CD117), WHO classification

Abstract
Thymoma is the most common anterior mediastinal tumor in adults and is frequently associated with autoimmune disorders such as myasthenia gravis. Thymomas are a diverse group of epithelial neoplasms with a behavioral spectrum that spans the complete clinical gamut from entirely benign to highly aggressive, lethal thymic carcinomas. The biologic behavior seems to depend primarily on the clinical stage at presentation and histologic subtype. This article discusses thymic organogenesis, Masaoka staging, WHO histologic classification of thymoma and thymic carcinoma, and selected molecular characteristics that highlight this diversity. This discussion will further underscore both the similarities and differences between categories of thymic epithelial neoplasms and offer support for the notion that tumor heterogeneity and/or tumor progression may explain the observed clinical variation in behavior. Recommendations are offered for future investigational approaches to further the understanding of the complexity of these tumors. (JNCCN 2010;8:1322–1328)

Tumors of the thymus are rare, accounting for 0.2% to 1.5% of all malignancies. Thymomas account for 15% to 20% of all mediastinal tumors and are the most common anterior mediastinal tumor in adults, with a relatively equal sex distribution.1-4 Thymoma in children is extremely rare. Most tumors are indolent, slow-growing, and relatively asymptomatic. Symptoms, if present, include chest pain, cough, pneumonia, phrenic nerve paralysis, or superior vena cava syndrome. Thymomas grow through local extension, and metastases, when present, include pleural, pericardial, or diaphragm invasion. Late metastatic sites include the lung, liver, and distant visceral sites. The association of thymoma with myasthenia gravis (MG) is well documented, and seen in 25% to 40% of patients with thymoma. A spectrum of other autoimmune disorders of neuromuscular, hematologic, dermatologic, endocrine, renal, or hepatic origin has been described.5

Thymic carcinomas are virtually never associated with autoimmune disorders.6 Patients with thymoma have a well-documented risk of second, unrelated malignancies with an incidence as high as 22%. Second malignancies include carcinoma of the stomach, liver, lung, uterine cervix, and head and neck and soft tissue tumors.7 The risk of second malignancies in patients without MG may be higher than in those with MG, suggesting inherent immune differences between tumor surveillance and autoimmunity.5 Thymoma is not unique to man, but also has been described in a dog, cats, dairy goats, small laboratory rodents, and a cow.9,10

Developmental Embryology and Functional Microanatomy

The spectrum of benign and malignant mediastinal neoplasms including thymoma, thymic carcinoma, germ cell tumors, malignant lymphoma, and neuroendocrine tumors is explained by thymic organogenesis. Developmental abnormalities including thymic hypoplasia, dysplasia, or atrophy lead to a variety of immunodeficiency disorders.11
Thymogenesis commences during the sixth week of gestation, with cellular contributions from the third and fourth pharyngeal pouches. The thymic primordium is populated by mesoderm, ectoderm, and endoderm, with the endoderm of the third pharyngeal pouch contributing the epithelial elements. The mesoderm contributes vascular stroma and mesenchymal cells, whereas the ectoderm envelopes the endodermal derived epithelium in a basement membrane–like manner. Thymocytes, the lymphoid component, are derived from fetal liver and bone marrow. Other cell types, including macrophages, B cells, interdigitating dendritic cells, Langerhans cells, mast cells, and neuroendocrine cells, complete the organ. Thymic compartmentation begins by week 10 of gestation with development of a lobular architecture, with each lobule having a well-defined cortex and medulla. Compartmentation is completed by week 16. The newly formed encapsulated thymus continues its caudal migration to the anterosuperior mediastinum and continues to grow postnatally through puberty, reaching a maximum weight of 30 to 40 g. Involution commences during early adulthood, with an increase in adipose tissue.

T-cell maturation continues throughout adult life, albeit at a diminished rate. The cortex is rich in immature lymphocytes (thymocytes; TdT- and CD5-positive) and epithelial cells, whereas the medulla becomes densely populated by the thymic epithelium, as identified by Hassall’s corpuscles. The epithelial cells express cytokeratin-intermediate filaments and are human leukocyte antigen–DR (HLA-DR)–positive and play a critical role in T-cell ontogeny. The perivascular space is extrathymic and analogous to a peripheral lymphoid organ. The thymus is the prototypical lymphoepithelial organ in which a finely controlled microenvironment provides the structural framework for generation of mature T cells. Autoreactive T cells are deleted by both negative and positive selection requiring cross-talk between lymphoid precursors and the thymic epithelium, which coexpresses HLA class II major histocompatibility complex (MHC) molecules and CD74. Benign thymomas have diminished coexpression of these molecules compared to invasive thymomas or thymic carcinoma. Experimental evidence in mice show that intrathymic epithelial precursors have the capacity to populate either cortex or medulla, and that these 2 compartments may be distinct.

Staging and Histologic Classification

Masaoka et al. early clinical staging criteria (Table 1) was built on earlier studies by Bergh et al. and Wilkins and Castleman. It focused on the integrity of the thymic capsule, macro- or microscopic invasion into adjacent structures, and metastasis. Masaoka’s original work excluded anaplastic carcinoma. Completeness of surgical resection was considered by Masaoka but was not analyzed as an independent variable for survival. Masaoka staging criterion has been independently verified by several studies as predictive of survival. Early studies by Blumberg et al., however, suggested that Masaoka clinical staging may not be applicable to thymic carcinomas (type C, WHO 1999) compared with other thymic histologies. Some investigators have suggested the collapse of stage I and II into one group because no significant difference was observed between stage I and II, in contrast to stage III or IV, which are distinctly different.

Although Masaoka’s original paper discussed histologic subtyping based on epithelial cell morphology and lymphocytic component, tumor histology has not been incorporated into staging systems. In fact, current NCCN Guidelines do not mention histology in staging, and no traditional TNM staging classification is recommended by the American Joint Committee on Cancer (AJCC). An extrapolation of the Masaoka system based on extent of invasion has been adapted to the TNM format (Table 1).

The histologic classification of thymoma as reviewed by Bernatz et al. has evolved significantly since the description by Symmers in 1932 in which 5 thymoma types were described: perithelioma (hemangiopericytoma), lymphosarcoma (lymphoma), epithelioma (epithelial tumor), spindle cell sarcoma (sarcoma), and Hodgkin disease. This classification, although imprecise by modern standards, highlights the diversity of thymic tumors. Bernatz et al. expanded the understanding of thymic tumors and were the first to describe the importance of capsular invasion and predominant cell type (epithelial or lymphocytic).

The histologic classification of thymoma has been a source of considerable controversy for several decades, with several classifications proposed. Universal adoption of a single classification has been hindered by the lack of common terminology among systems. Histologic reproducibility between observ-
ers also has been problematic. Previous classifications were based on recognition of thymic organotypic architecture,\textsuperscript{24,25} degree of differentiation,\textsuperscript{26,27} or presence or absence of capsular invasion with or without cytologic atypia.\textsuperscript{28}

Significant progress in the histologic classification of thymoma and thymic carcinoma was seen with the WHO expert panel consensus publication, first in 1999 and later updated in 2004,\textsuperscript{5} of a comprehensive classification. The WHO classification schema is based on thymic epithelial cell morphology (spindled/oval or round/epithelioid), degree of lymphocytic component, and presence or absence of epithelial atypia. Epithelial morphology corresponds to medullary or cortical differentiation. Tumors are categorized as type A, AB, B1, B2, B3, and C (thymic carcinoma) depending on the relative contributions of each cell type and the presence or absence of epithelial cell atypia (see Table 2). Reference to organotypic differentiation was omitted. The 1999 WHO type C category has been dropped in favor of the designation thymic carcinoma. For all practical purposes, thymic carcinomas are predominantly keratinizing squamous cell cancers, with other histologic types uncommon.\textsuperscript{29–37} Additional thymoma subtypes were added in the 2004 version with the recognition of metaplastic, micronodular with lymphoid stroma, microscopic, sclerosing, and lipofibroma types. Suster and Moran,\textsuperscript{26} however, suggested a simpler classification (thymoma, atypical thymoma, and thymic carcinoma) than the WHO consensus classification. They further observed that Masaoka staging and extent of resection (R0 vs. R1 vs. R2) were more important for management\textsuperscript{30} than histologic subtype.

Combined tumors with features of classic thymoma and thymic carcinoma have been described.\textsuperscript{38,39} In one series of 22 cases, the authors astutely postulate that given the microscopic juxtaposition of different histologies and transition from one type to another, thymoma and thymic carcinoma may represent a biologic spectrum rather than distinct entities.\textsuperscript{38}

For a classification system to be clinically useful it must be reproducible and predictive, offering prognostically useful information to the patient and clinician. The WHO histologic classification was proven to be prognostically useful in several studies\textsuperscript{40–49} and is thought to be independent of Masaoka stage. In general, types A, AB, and B1 have excellent overall disease-free survival rates typically greater than 90% to 95% at 10 years, with some investigators reporting 100% survival. However, overall survival rates for B3

<table>
<thead>
<tr>
<th>Stage</th>
<th>Extent of Invasion</th>
<th>TNM</th>
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<tbody>
<tr>
<td>I</td>
<td>Macro- and microscopically encapsulated, no invasion</td>
<td>T1 N0 M0</td>
</tr>
<tr>
<td>IIA</td>
<td>Macroscopic (transcapsular) invasion</td>
<td>T2 N0 M0</td>
</tr>
<tr>
<td>IIB</td>
<td>Microscopic transcapsular invasion of adjacent fat or mediastinal pleura, or grossly adherent to mediastinal pleura or pericardium</td>
<td>T3 N0 M0</td>
</tr>
<tr>
<td>III</td>
<td>Macroscopic invasion into adjacent organs (pericardium, great vessels, or lung)</td>
<td>T1-4 N0-N2 M0-M1</td>
</tr>
<tr>
<td>IVA</td>
<td>Pleural or pericardial dissemination</td>
<td>T4 N0 M0</td>
</tr>
<tr>
<td>IVB</td>
<td>Lymphatic or hematogenous dissemination</td>
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Table 2: WHO Histologic Subtype

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Epithelial Atypia</th>
<th>Predominant Cell Type</th>
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<tbody>
<tr>
<td>A</td>
<td>Spindle cell (medullary)</td>
<td>None</td>
<td>Epithelial</td>
</tr>
<tr>
<td>AB</td>
<td>Mixed thymoma</td>
<td>None</td>
<td>Distinct epithelial-rich and lymphoid cell-rich areas</td>
</tr>
<tr>
<td>B1</td>
<td>Lymphocyte rich, lymphocytic thymoma, predominantly cortical</td>
<td>None</td>
<td>Mixed</td>
</tr>
<tr>
<td>B2</td>
<td>Cortical thymoma (+/- anaplasia)</td>
<td>Mild to moderate</td>
<td>Increasing epithelial component</td>
</tr>
<tr>
<td>B3</td>
<td>Well-differentiated thymic carcinoma, epithelial thymoma</td>
<td>Increasing epithelial atypia</td>
<td>Predominantly epithelial, few lymphocytes</td>
</tr>
<tr>
<td>C</td>
<td>Thymic carcinoma</td>
<td>Overtly malignant</td>
<td>Epithelial</td>
</tr>
</tbody>
</table>
and C are typically poor, ranging from 20% to 70% and 0% to 30%, respectively. The broad survival range in B3 and C tumors may reflect imprecise histologic classification by pathologists or, more likely, tumor heterogeneity within each category.

Although both WHO classification and Masaoka stage are independent prognostic systems, there are little correlation between the two. It is unusual for type A tumors to be invasive (stage II–IV) because most are stage I, but it is also not uncommon for type C (thymic carcinoma) to be encapsulated (stage I). In a meta-analysis of 7 studies by Detterbeck,50 7% of all thymic carcinomas (type C) presented in stage I. Most, however, presented in either stage III (40%) or IV (46%). By contrast, 93% of type A thymomas present as either stage I (57%) or II (36%), with only 2% as stage IV. Although the data clearly identify preferential patterns at presentation, some degree of overlap exists among the categories.

**Molecular Pathogenesis**

Various techniques have been used to describe the molecular changes within each Masaoka stage or WHO histologic type. The data would suggest considerable overlap between categories. Molecular studies have been hampered by lack of established human thymic tumor cell lines. In certain lymphocyte-rich subtypes, the inability to separate the neoplastic epithelial cells from the nonneoplastic lymphoid component has been problematic. Investigators have used short-term primary cell culture or laser-assisted microdissection to generate epithelial cell–enriched material51 to circumvent this problem.

Perhaps of most interest, at least from a therapeutic standpoint, are studies of the epidermal growth factor receptor (EGFR) and the tyrosine kinase receptor, c-Kit (CD117). Studies of protein expression using immunohistochemistry have shown c-Kit overexpression in thymic carcinoma compared with thymoma.52–54 However, only a single case report has identified an activating c-Kit mutation55 that is the same as has been described in gastrointestinal stromal tumors (GIST). The reported patient initially responded to imatinib mesylate, but the effect was not durable. EGFR mutational status is similar to that seen in c-Kit.56,57 Although protein overexpression detected by immunohistochemistry has been described in a spectrum of WHO histologies and in invasive versus noninvasive tumors, no activating mutations in the tyrosine kinase domain have been identified. Significant correlation of EGFR expression with histologic subtypes or Masaoka stage has not yet been clarified, although invasive tumors seem to have a higher level of EGFR protein expression than noninvasive ones. The frequency of EGFR overexpression and intensity of expression in thymic carcinomas may be less than that seen in thymoma. Although no EGFR mutations have been described, Ionescu et al.58 identified gene amplification using fluorescence in situ hybridization (FISH) in thymoma and thymic carcinoma. B3 thymoma and type C thymic carcinomas had the highest gene copy numbers. They did not, however, identify a correlation between protein expression by immunohistochemistry and histologic type or Masaoka stage.

Several immunohistochemistry studies have focused on regulation of cell cycle by p53 and the cyclin-dependent kinase inhibitors p21 and p27.59,60 Baldi et al.,61 in a series of encapsulated WHO histologic types, showed that p53-positive and p21- and p27-negative tumors had the poorest disease-free survival. In their multivariate analysis, only p27 loss was significant. Zisis et al.62 confirmed that progressive loss of p27 correlates with WHO histology, with type B3 tumors having the lowest expression. Thymic carcinoma was not studied. Correlation of p27 loss with Masaoka stage was not entirely clear, particularly with higher-stage disease, because the sample number was limited (n = 2) in this study.

Hiroshima et al.61 reported increased proliferative index as measured by Ki-67 (Mib-1) labeling in type C and stage IV tumors compared with other types and lower-stage disease. The apoptotic index as determined through terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling assay, however, was slightly decreased in higher-grade tumors, thus yielding a net positive tumor growth fraction (cell proliferation > cell death).

Genetic aberrations and chromosomal imbalance in thymoma and thymic carcinoma have been investigated using various molecular techniques, including comparative genomic hybridization (CGH) for loss of heterozygosity (LOH), FISH, and microsatellite analysis.63–66 Genetic imbalances are common, being seen in 87% of cases studied by Inoue et al.67 Chromosome 6 deletions are the most common across all histologic types however are seen more fre-
quently in type B3 and C compared with type A.\textsuperscript{51} Type A tumors tend to be the most genetically homogenous with LOH on chromosome 6, including the 6p21.3, the MHC locus. Unusual type A cases that present at a higher stage have additional genetic abnormalities, including gains at 1q, 9q, 16, 17, and 20 and additional losses on 2q, 4q, 5q, 9p, and 13q.\textsuperscript{64} Common abnormalities in B3 and C tumors are gains at 1q and losses at 3p, 5q21 (APC locus), 13q13.3 (Rb locus), 16q, and 17p (p53 locus). All other WHO histologic types exhibit complex and heterogeneous abnormalities with increasing genetic instability in higher-grade tumors. However, considerable overlap exists among groups. An interesting subset of cases shares similar LOH despite divergent morphology and stage at presentation.\textsuperscript{62} For example, LOH at 5q15.3 in the APC locus has been seen in type AB thymomas in stage II tumors, type B2 in stages II and IVB tumors, and type B3 in stages III and IVA tumors. Similarities between some B2 and B3 subtypes suggest transition from a lower-grade to a higher-grade tumor. AB and B2 types that have been studied are complex and heterogeneous.\textsuperscript{62}

A recent approach by Lee et al.\textsuperscript{65} that used high throughput cDNA microarray–based CGH to identify genome wide alterations in 39 thymomas focused on deletions on chromosomes 1, 2, 3, 4, 5, 6, 8, 12, 13, and 18. Based on hierarchical clustering, the authors speculated that thymomas can be grouped into 4 distinct subgroups; A, AB, B1+2, and B3. Distinction between medullary and cortical epithelial differentiation was possible, and type AB tumors could be further classified as either type A or B, depending on the cluster analysis. Seventy genes seem to distinguish between type A and B3. Several of the informative genes that relate to cell structure and adhesion were deleted in B3 tumors.

**Summary and Future Directions**

Evidence is reasonably clear that thymic carcinoma is different from other thymoma types with a poorer prognosis, preferred metastatic sites, absence of associated autoimmune disorders, and a somewhat unique molecular signature. Do the data, however, justify separating thymic carcinoma from other thymoma types? An understanding of thymic organogenesis and tumor progression in the broadest biologic sense suggests that thymic epithelial neoplasms, as is seen in other organ systems, exhibit a broad spectrum of histologic patterns, biologic diversity, and clinical behavior.

The current understanding of thymoma classification suggests that aggressive behavior correlates to some degree with increasing epithelial cell abnormalities, both at the cytomorphologic level, with progressive loss of normal thymic organotypic architecture, and at the molecular level, with increasing genetic aberrations being seen in higher-grade tumors. However, considerable genetic and behavioral heterogeneity and overlap exist among the WHO classes and within each category. No single parameter clearly separates the categories. A more biologically precise theory based on the published observations would suggest that thymic carcinoma and B3 thymomas exist at the aggressive end of the biologic spectrum, whereas type A and AB tumors occupy the polar extreme, with benign behavior and virtually no thymoma-associated deaths. Intermediate behavior is seen in the other “bioactive” \(B\) types (B1 and B2).

The published data regarding Masaoka stage suggest that invasion through the thymic capsule into adjacent structures may be the biologically important determinant of behavior. One could speculate that transcapsular invasion might be analogous to basement membrane invasion, as is seen in progression from in situ to invasive disease in other cancers (i.e., colorectal, breast, melanoma). Theoretically, the biologic potential of in situ disease (Tis) is thought to be negligible because the fully evolved malignant phenotype capable of metastasis has not yet developed. In TNM staging of most other organs, depth of invasion (i.e., through the basement membrane or into adjacent structures) and/or tumor size are the primary determinants of \(T\) stage. Direct evidence supporting the notion that the thymic capsule is a functional basement membrane is lacking, but the biologic similarities are intriguing.

Given the biologic complexities identified in thymoma and thymic carcinoma, future management of patients with thymoma may be based on an assignment of biologic risk similar to that used GIST. A tumor would be regarded as low, intermediate, or high risk for recurrence and death depending on WHO histology, Masaoka stage, resection status (R0, R1, or R2), size, and perhaps the molecular signature.\textsuperscript{66}

Given the rarity of these tumors, clinical trials will require at least national, if not international, collaboration. Access to well-characterized, cryopre-
served, or formalin-fixed paraffin-embedded tissue for molecular interrogation will expand understanding of these tumors. The molecular signature between and within groups, particularly between thymic carcinoma and other type-B thymomas, including B3, should become clearer. Cooperative group support and leadership are required for studies of this magnitude. Ultimately, identification of therapeutic targets from this schema may alter the rather dismal prognosis of some patients with thymoma and thymic carcinoma.

References


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