State of the Art of Genetic Alterations in Thymic Epithelial Tumors

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Abstract: The rapid advent of technology in recent years has resulted in a substantial increase in our knowledge of the molecular underpinnings of thymic epithelial tumors. In addition to previously described chromosomal aberrations and alterations in DNA methylation, genome sequencing has helped unravel hitherto unknown mutations in these tumors. Attempts are also being made to develop gene signatures to help in the identification of patients likely to benefit from adjuvant therapy. Some of the recently identified genetic alterations have the potential to serve as targets for biological therapy, thus opening newer avenues for treatment of thymic epithelial tumors and increasing the number of effective options for treatment of recurrent or refractory disease.

Key Words: Thymoma, Thymic carcinoma, Methylation, Chromosomal abnormalities, Gene expression, Sequencing, Gene signatures

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Thymic epithelial tumors (TETs) are rare mediastinal cancers that have traditionally been treated using a multimodality approach consisting of surgery, radiotherapy, and platinum-based chemotherapy.[1] Effective treatment options are limited for patients with recurrent disease. The World Health Organization (WHO) classification is the most widely used system for the histological classification of TETs.[2] Based on thymic epithelial cell morphology, the degree of thymocyte involvement and the extent of epithelial atypia TETs are classified into the following subtypes: A, AB, B1, B2, B3, and C.

Efforts such as The Cancer Genome Atlas (TCGA) research network have helped in providing a deep understanding of the molecular alterations associated with a variety of tumors.[3–7] In comparison, until recently, scant information was available on genomic changes associated with TETs. Major challenges in obtaining genetic data still exist, especially from the more common thymoma subtypes (AB, B1, and B2) due to the presence of a significant number of non-neoplastic thymocytes. The latter typically outnumber and tightly intermingle with the neoplastic epithelial cells and largely preclude classical or array-based comparative genomic hybridization (CGH) approaches except in rare cases in which epithelial content is unusually high or in which thymocytes can be removed through short-term cell culture of neoplastic epithelial cells.[8–11] Fluorescence in situ hybridization analyses have been sparse as well.[8,12,13] However, with the advent of newer and more sensitive techniques in molecular biology (e.g., next generation sequencing) and bioinformatics, there has been and will be an incremental increase in the availability of high-quality data that has helped in expanding our understanding of the molecular pathogenesis of these diseases. Development of newer therapies based on an understanding of genomic alterations could potentially revolutionize treatment of TETs and result in an improvement in clinical outcomes. This review provides an overview of genetic alterations associated with TETs with a focus on recent discoveries that could have an impact on the management of thymic cancers.

CHROMOSOMAL ABNORMALITIES

Copy number (CN) gains and losses of various chromosomes have been well described in TETs, and the frequency of these changes is higher in the more aggressive histological subtypes. Based on the pattern of chromosomal changes, TETs can be clustered into various groups, demonstrating a correlation between genetic findings and WHO-based histology, with overlapping alterations between type AB and B2 thymoma, B2 and B3 thymoma, and B3 thymomas and thymic carcinomas.[8–11,14] In short, 6q25.2–25.3 loss is observed in all subtypes except B1 thymomas (a paucity of B1 thymomas among cases analyzed might have had a bearing on this result); 1q gains are common in type B2, B3 thymomas and thymic carcinomas; in addition, thymic carcinomas present with 1q, 4, 5, 7, 8, 9q, 12, 15, 17q, 18, 20 gains, and 3p, 6, 6p23, 9p, 13q, 14, 16q, 17p losses.[9–11,14] The biological significance of these changes is yet to be determined in the vast majority of cases. However, it is known that the 6p23 region encompasses the *FOXC1* tumor suppressor gene, and chromosomal loss is correlated with lower protein expression. Patients with *FOXC1*-negative tumors had a shorter time to progression and a trend for a shorter disease-related survival.[15]

ALTERATIONS IN DNA METHYLATION

Silencing of tumor suppressor genes such as *FHIT*, *MLH1*, and *E-cad* by promoter hypermethylation has been described in TETs.[16] These changes along with DNA hypomethylation have been correlated with Masaoka stage and

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WHO subtype of the TET. Other genes affected by aberrant methylation include *DAP-K*, *p16*, *MGMT*, and *HPP1*.[17] *MGMT* methylation and loss of its protein expression has been reported to be significantly more frequent in thymic carcinoma than in thymoma (74% versus 29%) and in advanced TETs (stages III/IV) compared with early stage tumors (stages I/II).[18] Methylation of the promoter region of *CDKN2* has been observed in up to 14% of thymomas and 25% of thymic carcinomas.[19] Immunohistochemical analysis of 132 TETs demonstrated CN loss of *CDKN2A* accompanied by lack of expression of its related protein p16INK4 and identified tumors with poor prognosis.[20]

A deeper understanding of alterations in DNA methylation can have important therapeutic implications. For example, *MGMT* hypermethylation is associated with a greater degree of sensitivity to alkylating agents.[21] Dysregulation of the cyclin-dependant kinase pathway can potentially be offset by using cyclin-dependent kinase inhibitors such as milciclib. An ongoing phase II study is evaluating the efficacy of milciclib in thymic carcinoma (NCT01011439). Forty-three patients have been enrolled and treated to date including 26 patients with thymic carcinoma and nine patients with B3 thymoma. Of 30 evaluable patients, 14 have met the prespecified primary end point of progression-free survival at 3 months (PFS-3 rate = 47%; 95% confidence interval [CI] 28.3–65.7%) including one partial response.[22]

GENOMIC PROFILING

Gene Expression Analysis

In an effort to overcome the limitations associated with traditional histological classification of TETs, efforts have been made to correlate the results of gene expression determined by whole genome gene expression analysis with clinical parameters such as stage of disease. Badve et al.[23] conducted a retrospective analysis in 34 patients with type AB and B thymoma using RNA extracted from fresh frozen tumors. Ten of 34 patients had received chemotherapy and eight patients had received radiation therapy. Eight patients were known to have metastatic disease either at diagnosis or developed it during the follow-up period. In addition, although all patients underwent surgery with curative intent, eight patients developed disease relapse during the follow-up period. After gene expression analysis using Partek Genomics Suite and Ingenuity Pathways Analysis, four molecular clusters of tumors were identified by unsupervised cluster analysis of 8260 genes that showed unique gene expression profiles. Supervised cluster analysis using 15 genes with the highest positive and negative fold changes helped in the identification of three clusters of histologically heterogeneous tumors. Analysis of clinical parameters such as stage of disease and presence of metastases did not show a correlation with either histological subtype or the newly identified gene expression clusters. However, ingenuity pathway analysis resulted in the identification of differentially expressed genes in patients with or without metastatic disease. In tumors obtained from the metastasis-positive group, there was a sixfold and threefold increase in the expression of *AKR1B10* and *JPH1*, respectively, whereas expression of *COL1A1* was decreased 13-fold. This is the first study to attempt a correlation between expression of individual genes and clinical factors in TETs. Validation of data obtained from this study has the potential to identify newer therapeutics targets such as *AKR1B10*.[24]

Gene Signatures

Traditional prognostic factors associated with TETs have included Masaoka stage, histological subtype, and the completeness of surgical resection.[25] Most attempts to identify prognostic and predictive biomarkers have focused on correlations with the stage and histological subtype of disease.[9,26–28] The first study to attempt a correlation between gene expression and the risk of development of metastases was reported in 2013.[29] In a retrospective study using archival samples of thymomas from multiple institutions, gene expression levels were used to categorize tumors into two groups based on the probability of developing metastases. This study included 111 cases of thymoma (36 in the training set and 75 in the validation set). Based on the prediction analysis of microarrays (PAMs), 19 differentially expressed genes and four reference genes were selected for quantitative reverse transcriptase polymerase chain reaction analysis. The primary end point of the study was to determine whether a gene signature could predict 5- and 10-year metastasis-free survival (MFS). A nine-gene signature was identified that could identify patients at low- and high-risk for metastasis with 5- and 10-year MFS of 77% and 26% ($p = 0.0047$) and 97% and 30% ($p = 0.0004$) in the training and validation sets, respectively. In univariate Cox regression analysis, the nine-gene signature was a strong predictor of the development of metastasis with a hazard ratio (HR) of 8.33 (95% CI = 1.99–34.9; $p = 0.004$) compared with a HR of 2.51 (95% CI = 1.22–5.15; $p = 0.012$) for presence of residual disease and a HR of 2.09 (95% CI = 0.95–4.59; $p = 0.067$) for Masaoka stage. In multivariate analysis, the nine-gene signature was the only independent predictor of the risk of developing metastasis (HR 5.26, 95% CI = 1.12–24.8; $p = 0.036$). Genes with increased expression in tumors with a higher metastatic potential included *AKR1B10*, *JPH1*, and *NGB*. Conversely, genes with decreased expression in tumors with high metastatic potential included *DACT3*, *SLC9A2*, *PDGFRL*, *FCGBP*, *PRRX1*, and *SERPINF1*.

The practical implications of development of prognostic biomarkers such as the gene signature illustrated above are the identification of patients at high risk for recurrence who might benefit from administration of appropriate adjuvant therapy and the potential to identify novel drug targets for patients at higher risk for development of unresectable, metastatic disease. However, significant challenges need to be overcome before widespread adoption of gene signatures in clinical practice, especially for rare diseases like TETs. These include the need for validation of results in a prospective setting, a clear demonstration of benefit over widely used clinical parameters such as Masaoka stage, histological subtype and completeness of resection, and the ease of performing the gene expression assay. Some of the limitations that might otherwise preclude mass validation of a gene signature for TETs can be surmounted by performing prospective trials involving