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Expression of mesothelin in thymic carcinoma and its potential therapeutic significance

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ABSTRACT

Objectives: Advanced thymic epithelial tumors (TETs) lack adequate treatment options in part due to absence of well characterized tumor-specific antigens. Mesothelin, a cell surface antigen, has been used successfully as a target for tumor-directed therapy. We sought to determine tumor expression and serum levels of mesothelin in patients with TETs.

Patients and methods: Tissue samples were obtained from 71 patients with histologically confirmed, unresectable advanced TETs and evaluated for mesothelin expression by immunohistochemistry. The evaluation was blinded for clinical data and outcome. Mesothelin expression and its association with clinico-pathological parameters and survival were assessed.

Results: Thymic carcinoma, thymoma, and thymic neuroendocrine tumors (NETs) accounted for 34 (48%), 29 (41%), and 8 (11%) cases respectively. Mesothelin expression was seen in a significantly larger proportion of thymic carcinoma (27/34, 79%) than thymoma (3/29, 10%) ($P < 0.0001$) and was absent in thymic NETs. Among thymic carcinomas 13/34 (38%) showed expression in nearly all tumor cells. Immunoreactivity was membranous, strong, and homogenous. Patients with thymic carcinoma and high mesothelin expression (in >50% of tumor cells) had significantly improved overall survival (median not reached, n = 19) compared to patients with no or low mesothelin expression (1.60 years; 95% CI: 1.24–4.94 years; n = 15; HR = 4.46, 95% CI: 1.55–12.80; p = 0.0026).

Conclusion: Mesothelin expression is frequently observed in advanced thymic carcinomas, infrequently in thymomas and is absent in thymic NETs. Due to strong, membranous expression mesothelin is a potential therapeutic target in thymic carcinoma.

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1. Introduction

Although the overall prevalence of thymic epithelial tumors (TETs) is very low, they are the most common cause of anterior mediastinal tumors in adults. The World Health Organization (WHO) system, which subdivides thymic epithelial tumors into thymoma subtypes A, AB, B1, B2, and B3 and thymic carcinomas is widely used to classify TETs based on their histologic appearance [1]. The histologic appearance correlates with the biology of the tumor and its prognosis [2]. Other important prognostic factors include the stage of the disease and the completeness of surgical resection.

No standard systemic treatments exist for relapsed or refractory TETs. In phase II trials of experimental agents, response rates have been low and progression-free survival limited [3]. The lack of effective therapeutic options is particularly obvious in thymic carcinoma. Less than a quarter of patients with inoperable thymic

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Table 1
Patient characteristics.

	N (%)
Age, Median (range)	51 (20–86)
Sex	
Male	38 (54)
Female	33 (46)
Race	
White	52 (73)
Asian	8 (11)
Black	7 (10)
Hispanic	3 (4)
Mixed	1 (1)
Histology	
Thymoma	29 (41)
AB	3
B1	2
B2	13
B3	11
Thymic carcinoma	34 (48)
Squamous, non-keratinizing	29
Squamous, keratinizing	1
Basaloid	1
Uncategorized	3
Thymic neuroendocrine tumors	8 (11)
Atypical carcinoid	2
Large cell neuroendocrine carcinoma	6
Stage at presentation	
II	1 (1)
III	4 (6)
IVA	15 (21)
IVB	51 (72)

carcinoma are alive 5 years after diagnosis [4]. Unlike many other epithelial tumors, tumor DNA sequencing has not identified targetable genes in TETs [5]. There is an unmet need to develop new and effective therapies for TETs, in particular for thymic carcinoma.

Mesothelin is a cell surface antigen that is present on normal mesothelial cells lining the pleura, peritoneum and pericardium [6]. Mesothelin is highly expressed in several cancers, including epithelioid mesotheliomas, pancreatic and biliary adenocarcinomas, gastric and ovarian cancers [7–10]. Several drugs targeting mesothelin are in various phases of clinical evaluation. They aim to exploit the differential expression of mesothelin in cancers compared with normal tissue [11–13]. Only two previous studies have assessed mesothelin expression in TETs wherein it was used as an immunohistochemical (IHC) marker for pathological differential diagnosis [14,15]. These studies were retrospective in nature, had a limited number of samples, did not provide clinical information and did not study the patterns of expression in detail. Given the paucity of data available, we sought to determine the expression patterns and prognostic value of mesothelin in TETs and the association of mesothelin expression in tumor cells with serum mesothelin, clinico-pathologic variables and survival.

2. Patients and methods

2.1. Patients

Tissue samples from patients with histologically confirmed, unresectable advanced (Masaoka stage III or stage IV) [16] TETs enrolled in clinical trials at the National Cancer Institute (NCI) between December 2007 and December 2012 (ClinicalTrials.gov Identifier: NCT 00965250, NCT00589290, NCT01100944, NCT01621568) [3,17–19] were included in this analysis. Patients who did not have tumor samples available for mesothelin IHC were excluded. Clinical and radiographic evidence was consistent with a diagnosis of a TET in all cases and there was no evidence to support a diagnosis of an alternative primary tumor source in any patient included in this study. All patients were followed for survival. All patients gave written informed consent in accordance with the NCI institutional review board regulations.

2.2. Tumor pathology evaluation

Tumor samples consisting of biopsy or resection specimens were obtained either from referring institutions or from procedures performed at the NCI and evaluated by the Laboratory of Pathology, NCI. Routine histopathological analysis was performed and tumors were subtyped according to the World Health Organization histological classification of TETs [1]. For the purposes of this analysis tumors were categorized into three groups: thymoma, thymic carcinoma and thymic neuroendocrine tumors (NETs). Although thymic NETs are included under the category of thymic carcinomas in the WHO classification [1] we separated them into an independent category based on previous literature showing an absence of mesothelin expression in small cell carcinoma and carcinoids [8,20]. All but one of the thymic carcinomas tested (19/20) were positive for CD5, or KIT (15/16), with all tested cases positive for at least one of these markers.

2.3. Tumor mesothelin expression

Mesothelin IHC was performed on tumor samples using monoclonal antibody 5B2 (Novocastra/Leica, Bannockburn, IL) at a dilution of 1:40. Incubation with primary antibody was preceded by 20 min heat-induced epitope retrieval in citrate buffer, pH 6.0. The detection was performed with Ventana Ultra View detection kit with DAB as the chromogen.

Immunohistochemical staining was evaluated by a pathologist (MM) with expertise in IHC who was blinded to the clinical data. The positivity (strength of labeling) was assessed as negative (no labeling), or positive, and the percentage of positive cells was also estimated.

2.4. Serum mesothelin

Mesothelin (nmol/L) was measured in serum using the MesomarkTM (Fujirebio Diagnostics, Inc., Malvern, PA). Assays were run according to manufacturer's instructions, blinded to patient data. The normal level of serum mesothelin is $\leq$1.5 nM/L.

2.5. Statistical methods

The association of gender with mesothelin positivity was determined using Fisher's exact test. The association of race and histology with mesothelin positivity was determined by Mehta's modification to Fisher's exact test, [21] while a Cochran-Armitage test for trend was used to assess the association between stage and mesothelin positivity [22]. The Kruskal-Wallis test was used to compare distributions of mesothelin expression (%) according to histology. Overall survival was defined as the time from date of diagnosis of metastatic cancer to date of death or last follow-up. The association between mesothelin expression or histology and survival was estimated using the Kaplan-Meier method, with curves compared using a log-rank test. Hazard ratios and associated confidence intervals were determined using a Cox proportional hazards model. All p-values are two-tailed and presented without adjustment for multiple comparisons. Univariate survival analyses were performed on the following prognostic factors: age, sex, race, stage and histology. Log-rank test and trend test p-values were calculated as appropriate. Prognostic factors associated with survival were subsequently included in a multiple proportional hazards