Prognostic Molecular Subtypes of Low-Grade Cancer of the Appendix

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BACKGROUND: Appendiceal cancer (AC) patients treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) often demonstrate an unpredictable variability in their survival outcomes. Biomarkers predictive of CRS/HIPEC efficacy could better guide treatment decisions. We hypothesized that variation in the transcriptional programming of AC tumors might distinguish molecular subtypes with differential outcomes after CRS/HIPEC.

STUDY DESIGN: Gene expression profiles of 2 AC cohorts were analyzed using Affymetrix whole-genome expression microarrays. Hierarchical clustering methods, Kaplan-Meier analysis, and Cox regression models were used to discover and validate prognostic molecular subtypes of AC. Gene set enrichment analysis was used to infer pathologic attributes of the molecular subtypes.

RESULTS: Unsupervised hierarchical clustering analysis of tumor expression profiles revealed a 139-gene cassette that distinguished 2 molecular subtypes (based on low vs high expression of the gene cassette) with statistically significant survival differences (disease-specific survival, $p = 0.0075$; progression-free survival, $p = 0.0072$). In a second AC cohort, the 139-gene cassette reproducibly partitioned tumors into subtypes with significant survival differences. Tumors showing high relative expression of the genes comprising the cassette associated with poor survival outcomes (disease-specific survival, $p = 0.047$; progression-free survival, $p = 0.0079$), and exhibited gene expression patterns enriched for oncogenic processes and pathways. The prognostic value of the molecular subtypes was specific for low-grade appendiceal tumors (disease-specific survival, $p = 0.028$; progression-free survival, $p = 0.0016$), and remained significant in the presence of conventional prognostic markers, including grade, surgical resection score, Eastern Cooperative Oncology Group status, and age.

CONCLUSIONS: The 139-gene cassette can have actionable clinical utility for identifying low-grade appendiceal tumor molecular subtypes predictive of therapeutic efficacy of CRS/HIPEC. (J Am Coll Surg 2016;222:493–503. © 2016 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

It is estimated that approximately 1% of all appendectomy specimens will contain a neoplasm.[1] The most common cancers of the appendix are neuroendocrine tumors (carcinoid), benign mucoceles, and mucinous carcinoma. Appendiceal cancer (AC) is a rare disease, yet its incidence in the reported literature varies, depending on the histologic types included in the classification of appendiceal malignancies.[2,3] In a Surveillance, Epidemiology, and

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Abbreviations and Acronyms

AC = appendiceal cancer
CRS = cytoreductive surgery
DSS = disease-specific survival
GSEA = Gene Set Enrichment Analysis
HIPEC = hyperthermic intraperitoneal chemotherapy
LFC = log fold change
LGA = low-grade appendiceal
PFS = progression-free survival
PSD = peritoneal surface disease
RNA = ribonucleic acid

End Results database retrospective analysis, that excluded low-grade carcinoid tumors, the annual age-adjusted incidence of appendiceal primaries was 0.12 cases per 1,000,000 of population. Appendiceal adenocarcinoma represented 66.5% of these patients.[3] Extrapolating from the fact that the Surveillance, Epidemiology, and End Results program collects data from 14% of the US population, the annual incidence of appendiceal adenocarcinoma in the country should be around 300 to 400 cases, although estimates up to 3,500 cases annually in the United States have been made.[4] The rate of appendiceal neoplasms is believed to have increased by >50% since the turn of the century.[4]

Although rare, AC is associated with considerable mortality due to the late stage at diagnosis and the low likelihood of it being found on screening colonoscopic examinations.[5] Mucinous ACs rupture all too frequently, leading to peritoneal surface disease (PSD) or so-called "carcinomatosis." Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) is an established modality for the treatment of peritoneal dissemination from appendiceal tumors,[6-13] as well as a variety of epithelial primaries. Survival after CRS/HIPEC for appendiceal neoplasms with PSD is multifactorial and often depends on tumor biology, volume of disease at presentation, completeness of CRS, and patient's functional status and comorbidities.[7-12]

Patients with PSD from low-grade appendiceal (LGA) primaries have traditionally been considered the best candidates for CRS and HIPEC, primarily due to favorable biologic behavior characterized by a predominant pattern of late or noninvasive superficial spread into tissues, with minimal risk of hematogenous dissemination.[8,9,12] However, even within the LGA group, clinical outcomes, such as progression-free survival (PFS) and disease-specific survival (DSS), show a significant and often unpredictable variability.[7-12] This variability is greater when the studied cohorts include patients with high-grade appendiceal primaries, even when the extent of disease and completion of CRS are factored into the survival analysis.[12]

Two well-accepted light microscopy-based histologic classification systems have only partially stratified the polymorphic and often convoluted clinical spectrum of PSD from appendiceal primaries.[8,13] Both systems use a combination of features, including presence of mucin and epithelium, cytologic atypia, degree of proliferation, architectural complexity, mitotic activity and parenchymal invasion. The Ronnett system[13] identified 3 tiers of tumor histology with prognostic significance: disseminated peritoneal adenomucinosis, peritoneal mucinous carcinomatosis with intermediate or discordant features, and peritoneal mucinous carcinomatosis. On the other hand, the Bradley system[8] combined the intermediate and the low categories together and came up with a 2-tier system: mucinous carcinoma peritonei low grade and high grade. Although the 2 histologic classification systems are each significant and reliable for predicting prognosis, even in the good prognostic subgroup, there is a substantial failure rate despite complete cytoreduction.

In previous work, we investigated the genome-wide gene expression profiles of a panel of peritoneal metastases comprising 26 appendiceal and 15 colorectal tumors.[14] Through unsupervised clustering analysis, 3 distinct tumor subclusters of mixed histologies were identified. Kaplan-Meier analysis demonstrated that the tumor subclusters, composed of both appendiceal and colorectal tumors, were prognostic in nature, stratifying patients into significantly different survival groups with potential for clinical impact, particularly in the context of low-grade appendiceal disease.

In the current study, we sought to more fully characterize the transcriptome of AC, focusing on the global gene expression patterns and transcriptional subsets of genes that associate with patient prognosis. We hypothesized that the clinical diversity in patient outcomes could be resolved, in part, through the identification of differences in tumor transcriptional programming that distinguish more and less aggressive disease subtypes. In this work, we discovered and validated the existence of 2 distinct prognostic subtypes most applicable to LGA tumors, as differentiated by a 139-gene transcriptional cassette, and demonstrated the additive prognostic value of these novel subtypes in comparison with conventional predictive variables.

METHODS

Patients and clinical characteristics

Selection of AC cases was facilitated by an IRB-approved, prospectively maintained database of clinical and