Alternative Lengthening of Telomeres Predicts Site of Origin in Neuroendocrine Tumor Liver Metastases

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BACKGROUND: The determination of the primary tumor origin in patients with neuroendocrine tumor liver metastases (NELM) can pose a considerable management challenge. Recent studies have shown that the alternative lengthening of telomeres (ALT) is prevalent in some human tumors, including pancreatic neuroendocrine tumors (PanNET), and can be useful in predicting tumor biology. In this study, we aimed to evaluate the use of ALT as a biomarker in patients with NELM, in particular to predict the site of origin of metastases.

METHODS: Tissue microarrays (TMAs) were constructed using tumor tissue from NELM patients undergoing liver resection between 1998 and 2010. These included 43 PanNET and 47 gastrointestinal carcinoid tumors. The TMAs were tested for ALT using telomere-specific fluorescent in situ hybridization. The association between ALT positivity and clinicopathologic features and long-term outcomes was investigated.

RESULTS: Alternative lengthening of telomeres was positive (ALT+) in 26 (29%) of the 90 tumors included in the TMAs. Pancreatic neuroendocrine tumors were ALT+ in 56% of patients, compared with only 4% ALT+ among gastrointestinal carcinoid tumors ($p < 0.001$). The specificity of ALT for detecting pancreatic origin was 96% and the positive predictive value was 92%, and sensitivity was 56% and the negative predictive value was 70%. Additionally, ALT was associated with the pattern of metastatic disease: ALT+ NELM were more likely to have oligometastases ($p = 0.001$) and less likely to be bilateral in distribution ($p = 0.05$) than were ALT tumors. In addition, ALT+ was associated with improved prognosis in the PanNET patient population.

CONCLUSIONS: Alternative lengthening of telomeres was found to be a useful biomarker in patients with NELM. This marker can be helpful in guiding therapy by identifying the site of origin in patients in whom the primary site is unknown. (J Am Coll Surg 2014;218:628–635. © 2014 by the American College of Surgeons)

Neuroendocrine tumors (NETs) consist of a diverse group of neoplasms that exhibit substantial variation in biological behavior and response to treatment. Specifically, NETs of unknown primary site account for 10% to 13% of all NETs.[1,2] They often manifest as neuroendocrine liver metastases (NELM), discovered with nonspecific symptoms or incidentally during abdominal imaging performed for other indications. The diagnosis of NET of unknown primary is typically established after fine-needle aspiration biopsy of the liver metastases reveals neuroendocrine differentiation on light microscopy and immunohistochemical staining. In the majority of those cases, the primary NET will be either pancreatic neuroendocrine tumor (PanNET) or gastrointestinal (GI) carcinoid tumor.[3-5] Extensive imaging with CT, MRI, somatostatin receptor scintigraphy, and PET is often used in an attempt to identify the primary tumor. When resectable, exploratory laparotomy combined with hepatectomy and search for the occult primary is generally recommended when preoperative evaluation

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Abbreviations and Acronyms

ALT = alternative lengthening of telomeres
FISH = fluorescence in situ hybridization
GI = gastrointestinal
HR = hazard ratio
NET = neuroendocrine tumor
NELM = neuroendocrine liver metastases
OS = overall survival
PanNET = pancreatic neuroendocrine tumor
TMA = tissue microarray

fails to establish a site of origin.[6,7] When unresectable, in the event that all attempts to identify the primary tumor fail, patients are usually treated empirically with hormonal or cytotoxic chemotherapy based on the liver metastases' histologic grade and the presence of symptoms. However, determination of the primary site is becoming increasingly important. Specifically, advances in the understanding of the genetics of PanNET have identified unique molecular features of these tumors, some of which, such as mammalian target of rapamycin pathway mutations, are potentially targetable.[8] Newer targeted therapies have been shown to be effective in patients with PanNET, including everolimus and sunitinib.[9-11] Therefore, the benefit of identifying a molecular biomarker in NET that can help determine the primary tumor site from a biopsy of NELM would be clinically useful. Recent studies have demonstrated an uncommon but distinct pathway in which neoplastic cells can overcome the chromosome end replication problem and gain immortality. Although most neoplasms rely on the upregulation of telomerase to maintain telomere length, approximately 5% of human tumors appear to use a telomerase-independent pathway, called the alternative lengthening of telomeres (ALT) mechanism.[12] The ALT was originally described in human sarcomas and astrocytomas, but has since been found to be prevalent in a variety of human malignancies, including NET.[13] Early studies have suggested that this biomarker can be useful in predicting tumor biology. Our aim in this study was to evaluate the use of ALT as a biomarker in patients with NELM. In particular, we aimed to determine if ALT can be useful in predicting the site of origin of the NET metastases.

METHODS

Identification of patients and construction of the tissue microarrays

All patients who underwent liver resection for NELM between 1998 and 2010 at the Johns Hopkins Hospital were identified after approval of the study by the Institutional Review Board. The pathology database was evaluated for the availability of formalin-fixed, paraffin-embedded tumor tissue blocks of their liver metastases. The available tissue blocks were reviewed and marked by a pathologist (MT) to confirm the presence of tumor tissue. Core biopsies were obtained from the tumor-containing area of the blocks and used to construct tissue microarrays (TMAs) to facilitate immunolabeling and scoring. Three core biopsies from each tissue block were used in the TMAs in an effort to minimize the sampling error. Controls of normal human tissue cores were also incorporated in the TMAs, in a ratio of 1 control for every 3 tumor cores.

Hybridization and scoring for alternative lengthening of telomeres

The methodology used for telomere-specific fluorescence in situ hybridization (FISH) of specimens was as described by Heaphy and colleagues.[14] Briefly, deparaffinized TMAs slides were hydrated, steamed for 20 minutes in citrate buffer (catalog no. H-3300; Vector Laboratories), dehydrated, and hybridized with a Cy3-labeled peptide nucleic acid probe complementary to the mammalian telomere repeat sequence ([N-terminus to C-terminus] CCCTAACCCTAACCCTAA). As a positive control for hybridization efficiency, a fluorescein isothiocyanate–labeled peptide nucleic acid probe having specificity for human centromeric DNA repeats (ATTCGTTGGAAACGGGA; CENP-B binding sequence) was also included in the hybridization solution. After post-hybridization washes, slides were imaged with a Nikon 50i epifluorescence microscope equipped with X-Cite series 120 illuminator (EXFO Photonics Solutions Inc.) and appropriate fluorescence excitation/emission filters. Grayscale images were captured using Nikon NIS-Elements software and an attached Photometrics CoolsnapEZ digital camera, pseudo-colored and merged. Quantification from the digital images was conducted using Telometer, a custom software plugin created for the open source image analysis program ImageJ, freely available for download (http://bui2.win.ad.jhu.edu/telometer/).

The slides were scored by 2 independent reviewers (CH and AM) blinded to the tumor characteristics. In our study, NELM were classified as ALT+ if they met the following criteria: presence of ultra-bright, intra-nuclear foci of telomere FISH signals, with integrated total signal intensities for individual foci being >10-fold that of the per cell mean (integrated signal intensities for all telomeric signals in individual benign stromal cells within the same case) and ≥1% of neoplastic cells displaying ALT-associated telomeric DNA foci. Tumor samples lacking ALT-associated telomeric foci in which at least