Comparison of tumor markers for predicting outcomes after resection of nonfunctioning pancreatic neuroendocrine tumors

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Background. *This study compares the predictability of 5 tumor markers for distant metastasis and mortality in pancreatic neuroendocrine tumors (PNETs).*
Methods. *A total of 128 patients who underwent pancreatectomy for nonfunctioning PNETs between 1998 and 2011 were evaluated. Tumor specimens were stained via immunochemistry for cytoplasmic and nuclear survivin, cytokeratin 19 (CK19), c-KIT, and Ki67. Univariate and multivariate regression analyses and receiver operating characteristics curve were used to evaluate the predictive value of these markers.*
Results. *A total of 116 tumors (91%) were positive for cytoplasmic survivin, 95 (74%) for nuclear survivin, 85 (66.4%) for CK19, 3 for c-KIT, and 41 (32%) for Ki67 >3%. Twelve (9%) tumors expressed none of the markers. Survivin, CK19, and c-KIT had no substantial effect on distant metastasis or mortality. Age >55 years, grade 3 histology, distant metastasis, and Ki67 >3% were associated with mortality (P < .05). A cut-off of Ki67 >3% was the best predictor (83%) of mortality with an area under the curve of 0.85. Ki67 >3% also predicted occurrence of distant metastases with odds ratio of 9.22 and 95% confidence interval of 1.55–54.55 (P < .015).*
Conclusion. *Of the 5 markers studied, only Ki67 >3% was greatly associated with distant metastasis and death. Survivin, CK19, and c-KIT had no prognostic value in nonfunctioning PNETs. (Surgery 2014;156:1504-11.)*

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Despite recent advances in genetics and molecular biology, the behavior of pancreatic neuroendocrine tumors (PNETs) remains incompletely understood. Although functioning PNETs have a more predictable outcome, the clinical course of nonfunctioning PNETs is less certain. In the absence of distant metastasis, predicting malignant behavior of nonfunctioning PNETs remains a challenge. In our previous publication, we found that patients older than 55 years with nonfunctioning PNETs with grade 3 histology and distant metastasis had an increased risk of mortality.[1] Other factors, such as tumor size, lymph node involvement, tumor necrosis, and perineural invasion, have been reported to have prognostic value for these tumors in other series[2,3]; however, the reliability of these other prognostic factors has not been demonstrated consistently in all series, including our own.

In other tumors with variable behavior, intracellular tumor markers have been found to be reliable predictors of poor outcome. The use of these tumor markers in nonfunctioning PNETs has been attempted but has not been validated completely. In most studies, a single tumor marker has been evaluated for its ability to predict tumor

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behavior. To better determine the predictive value of tumor markers for the outcome of patients with PNETs, we studied a number of potential markers, including nuclear and intracellular survivin, cytokeratin-19 (CK19), c-KIT, and Ki67.

Survivin is an apoptosis inhibitor expressed in fetal tissue and is absent in normal, terminally differentiated tissues. In the cytoplasm, it inhibits apoptosis, but in the nucleus it regulates cell division. Survivin is unregulated in a variety of cancers and is overexpressed in PNETs.[4,5] CK19 is an intermediate filament protein found in the cytoplasm of epithelial tissue. In the adult pancreas, it is a marker of ductal cells. When present in islet cells, it suggests a poor outcome.[6,7] c-KIT is a tyrosine-kinase receptor found on the surface of the progenitor cells of the pancreas. Its expression in islet cells signifies immature, poorly differentiated tumors.[8] Ki67 is a marker of cellular proliferation that has prognostic value in several malignancies including PNETs.[9,10] We evaluated the expression of these different intracellular markers and their effect on metastasis and death in patients with nonfunctioning PNETs.

METHODS

The databases of NorthShore University Health System, Northwestern Feinberg School of Medicine/Jesse Brown VA Medical Center, University of Chicago Medical Center, and Rush University Medical Center were reviewed to identify patients who had pancreatectomy for nonfunctioning PNETs between 1998 and 2011. Institutional research board approval was obtained from each institution. Chart reviews were performed, and data were collected regarding patient demographics, mode of presentation, type of pancreatectomy, tumor size, histologic grade, lymph node involvement, lymphovascular or perineural invasion, and distant metastases. Table I summarizes the patient characteristics and pathologic evaluation.

Four-micrometer sections of representative formalin-fixed, paraffin-embedded tumor blocks were used for immunohistochemical staining using antibodies for survivin and Ki67 (Thermo Scientific Lab Vision, Fremont, CA), and CK19 (Biocare, Concord, CA) according to the manufacturer's directions. Specifically, survivin was used at a 1:50 dilution, CK19 at a 1:10 dilution, c-KIT at a 1:1,000 dilution, and Ki67 at a 1:50 dilution. Survivin, CK19, and c-KIT-stained sections were all reviewed by 2 pathologists blinded to patient outcome and tumor grade and scored according to methods used in previous publications.[5-7] Discrepancies in scoring were resolved by re-review by the senior pathologist. Nuclear and cytoplasmic staining for surviving were each categorized as being negative if there was <5% staining, focally positive if there was 5–50% staining, and diffusely positive if there was >50% staining. For CK19, results were tabulated according to the previously published method,[6] but ultimately, the specimens were considered to be either negative (0%), focally positive (<50%), or diffusely positive (>50%). For c-KIT, tumors with >5% staining were considered positive; positive cases were subdivided into those with >5–50% staining and those with >50% staining. For Ki67, the scoring was performed by the 2 pathologists using the technique detailed by Adsay.[11] Specifically, digital images of "hot spots" in stained sections captured at a magnification of 200× were printed out, and all tumor cells in the field were counted manually. The percentage of Ki67-positive cells was recorded.

Statistics. Univariate and multivariate analysis of prognostic factors were based on a Cox proportional hazards model. The effect of the expression of the tumor markers on death and metastasis was assessed via survival curves and estimates using the Kaplan-Meier method. Receiver operating characteristic (ROC) curve analysis was done to establish the cut off value for Ki67. The analysis was done using SAS statistical software version 9.0 (SAS Institute, Inc, Cary, NC).

RESULTS

Clinicopathologic characteristics. A total of 128 patients with an age (±SD) of 55 ± 14 years were included in this series. There were 71 males and 57 females. The median patient follow-up was 33 months. Forty-two patients (33%) had undergone a pancreatectomy, 78 (61%) had a distal pancreatectomy, 2 (1%) had an enucleation, and 6 (5%) had a central pancreatectomy. The tumor size was 3.3 ± 2.0 cm, and 56 patients (44%) had a tumor less than or equal to 2 cm. The tumor grade was based on mitotic rate and histologic appearance: 73 patients (57%) had grade 1 tumors, 37 (29%) had grade 2, and 18 (14%) had grade 3. Eighteen patients (14%) had liver metastases, and of these, 3 patients had a tumor less than 2 cm. The clinical and pathologic results have been reported in detail in our previous publication.[1]

Immunohistochemistry. *Survivin.* Cytoplasmic survivin was expressed either focally or diffusely in 116 tumors (91%). The other 12 tumors had less than 5% expression of this marker and were considered negative for survivin. There was no