

KIT is an Independent Prognostic Marker for Pancreatic Endocrine Tumors – a Finding Derived from Analysis of Islet Cell Differentiation Markers

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Abstract

Purpose: The biologic behavior of pancreatic endocrine tumors (PETs) is difficult to predict by histologic features alone. We sought to use molecular markers to predict the clinical behavior of PETs based on islet cell development from pancreatic precursor cells to mature islet cells.

Patients and Methods: Immunohistochemistry was performed in 97 PETs using antibodies to KIT, CK19, and Pax6. The associations between immunohistochemical features and survival were evaluated with Cox proportional hazards regression model. A three-tiered molecular grading system was developed according to the tumor immunohistochemical profile. Correlations between this system and tumor functional status, metastasis, recurrence, and survival were analyzed.

Results: Both KIT and CK19 expression were associated with survival, but only KIT was an independent prognostic marker. Mutations were not detected in KIT exons 9 and 11. A three-tiered molecular grading system was derived from combination of KIT and CK19 which includes: grade 1, KIT-/CK19- tumors; grade 2, KIT-/CK19+ tumors; and grade 3, KIT+/CK19+ tumors. Disease-specific survival rates at 10 years were 75.1%, 57.4%, and 10.4% respectively (p<0.001). The metastasis and recurrence rates were also significantly different among the three groups.

Conclusion: By evaluating the molecular pathways of islet cell differentiation in PET, a new and independent prognostic marker KIT was identified. Specific molecular markers related to islet cell differentiation may be used to determine the differentiation of PET. The molecular grading system derived with these markers was able to predict clinical behavior of PET.

Patients And Methods

Study population
 Ninety-seven patients were included in this study. Demographic information including age and gender was recorded. The functioning status of PETs was determined according to clinical presentations. The follow-up information including metastasis, recurrence, and tumor-specific death was collected. The duration of follow-up was calculated from the date of surgery to the date of recurrence, death, or last follow-up.

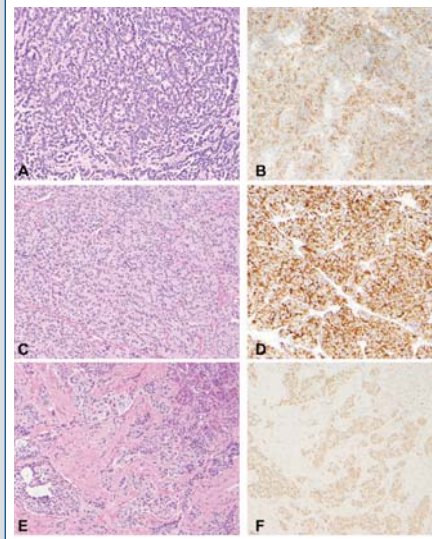
Immunohistochemistry
 Immunohistochemistry was performed using standard avidin-biotin complex (ABC)-peroxidase procedure with positive and negative controls. The immunostaining for KIT (rabbit polyclonal, 1:1000, DAKO, Carpinteria, CA), CK19 (mouse monoclonal, 1:20, clone RCK108, DAKO, Carpinteria, CA) and Pax6 (rabbit polyclonal, 1:100, Abcam, Cambridge, MA) was performed with an automated (DAKO) stainer.

DNA extraction and KIT mutation analysis
 DNA from 21 KIT+ PET FFPE blocks was extracted using a Qiagen extraction kit (Qiagen, Valencia, CA) and quantified by Nanodrop (ThermoFisher Scientific, Waltham, MA). Mutation analysis was performed by PCR-DHPLC analysis.

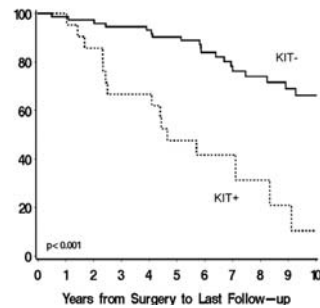
Statistical analysis
 Comparisons of clinicopathologic features by KIT/CK19 status were evaluated using Kruskal Wallis, chi-square, and Fisher's exact tests. Recurrence-free and disease-specific survivals were estimated using the Kaplan-Meier method. Associations of clinical, pathologic, and immunohistochemical results with recurrence and death from disease were evaluated using Cox proportional hazards regression models.

Immunohistochemical results

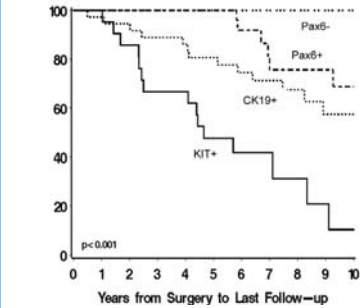
Twenty-two percent (21/97) of PETs were positive for KIT (A&B). Sixty percent (58/97) of PETs were immunoreactive to CK19 (C&D). A majority of PETs (73%, 70/96) were positive for Pax6 (E&F).



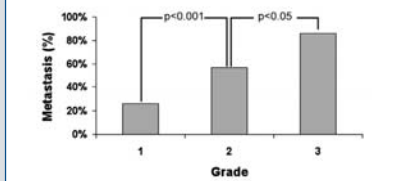
Disease-specific survival with regard to KIT immunoreactivity



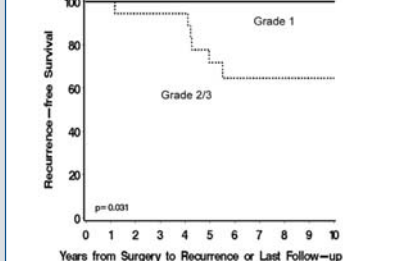
Disease-specific survival with regard to the differentiation model



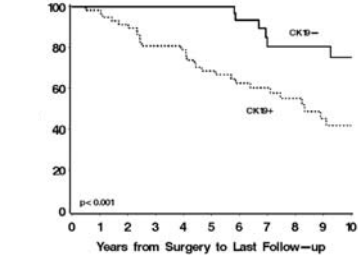
The metastasis rate in different grades of PETs



Recurrence-free survival with regard to three-tiered molecular grading system



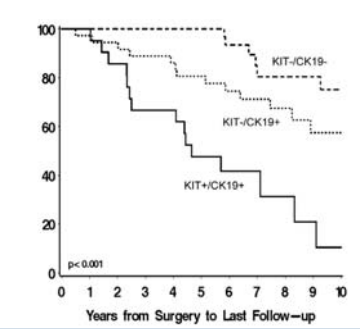
Disease specific survival with regard to CK19 immunoreactivity



A three-tiered molecular grading system correlated with metastasis, recurrence, and survival

Grade 1: KIT-/CK19-
 Grade 2: KIT-/CK19+
 Grade 3: KIT+/CK19+

Disease-specific survival with regard to three-tiered molecular grading system

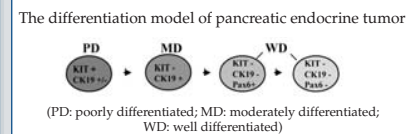


KIT is an independent prognostic marker

Table 2
 Univariate analysis of association of death with clinical and immunohistochemistry results in 97 PETs

Features	Risk Ratio (95% CI)	P-value
Age (10-year increase)	1.00 (0.78 - 1.27)	0.976
Sex		
Female		
Male	1.32 (0.69 - 2.51)	0.401
Functioning vs. non-functioning	0.45 (0.19 - 1.02)	0.057
Insulinomas vs. others	0.23 (0.05 - 0.94)	0.04
Positive CK19	4.08 (1.79 - 9.33)	<0.001
Positive KIT	5.21 (2.66 - 10.21)	<0.001
Positive Pax6	0.62 (0.32 - 1.21)	0.161

A differentiation model of PET derived from islet cell differentiation markers



Introduction

The biologic behavior of pancreatic endocrine tumors (PETs) is difficult to predict using histologic features alone. The most reliable evidence of malignant behavior in PETs is metastasis to regional lymph nodes or liver or gross infiltration of adjacent organs.

CK19 was shown to be a powerful prognostic marker of PETs though not all studies have found it to be an independent marker or even a good predictor of clinical behavior. The mechanism by which CK19 was able to be a valuable prognostic marker for PET remains unknown.

Since CK19 is only expressed in fetal islet cells, it may be a marker of islet cell early differentiation or immature PET cells. Our hypothesis was that PETs with stem cell-like phenotype are poorly differentiated and have the worst prognosis, while PETs not expressing stem cell markers show some degree of differentiation and would be associated with better prognosis.

The purpose of this study was to investigate the expression of islet cell differentiation markers including Kit, CK19 and Pax6 in PETs. Correlations with tumor-specific survival, metastasis, tumor recurrence, and tumor functioning status were analyzed at 10 years were 75.1%, 57.4%, and 10.4% respectively.

Results

Demographics, functioning status, and follow-up

Table 1
 General information and clinical outcomes of 97 PETs

Features	
Age (years); mean (median; range)	53.9 (54; 22 - 82)
Sex (F:M)	46:51
Functioning tumors	n=32 (34%)
Insulinoma	n=20
Glucagonoma	n=3
Gastrinoma	n=6
VIPoma	n=1
Ectopic ACTH producing tumor	n=2
Metastasis	n=49 (51%)
Recurrence	n=8 (8%)
Tumor-specific death	n=38 (39%)
Overall survival rates at 5 and 10 years (95% CI)	80.6% (72.9 - 89) and 54.5% (43.5 - 68.2)

Summary

Both KIT and CK19 expression were correlated with survival of PETs, but only KIT was an independent prognostic marker for PETs.

The degree of tumor differentiation can be determined according to these markers, with KIT representing a stem cell-like phenotype, CK19 representing early epithelial cell differentiation, and Pax6 reflecting subsequent endocrine differentiation.

The three-tiered molecular grading system derived from different expression patterns of KIT/CK19 predicted survival, metastasis, recurrence, and is also correlated with tumor functioning status.

Conclusion

By analysis of islet cell differentiation markers in PETs, we found that KIT is a new and independent prognostic marker for PETs. The three-tiered molecular grading system derived from islet cell differentiation model correlates with the clinical behavior. Similar strategies may be applicable to other tumor types to define new prognostic markers and to apply molecular grading as a more objective method of predicting tumor behavior.

References

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KIT mutations

No mutation for KIT exons 9 and 11 in 21 KIT+ PETs.