Pancreatic Neuroendocrine Tumor: Genetic Signatures

By

Dr. Huyen Pham

School of Medicine of Tan Tao University

Abstract

Pancreatic neuroendocrine tumors (PanNETs) are the second most common malignant tumor of the pancreas. Neuroendocrine tumors are neoplasms that arise from cells of the endocrine and nervous systems. They all share common features such as having special secretory granules and producing biogenic amines and polypeptide hormones. The mean age of PanNETs occurrence is of 58 years old, and although their prognosis are better than that of pancreatic ductal adenocarcinoma, they are still poor tumors with an average overall 5 years survival only 42%. PanNETs are well classified into functional versus non-functional tumors. While functional PanNETs are easily to be recognized by their classic clinical presentations such as Whipple's triad for insulinoma or the migratory necrolytic erythema for glucagonoma, patients with non-functional PanNETs usually do not suffer from symptoms due to hormone hypersecretion, even if hormone levels are elevated on laboratory evaluation. Although several TNM classification systems for PanNETs patients have been established, a recent study suggests that the ENETs (European Neuroendocrine Tumor Society) TNM classification was superior to the AJCC (American Joint Committee on Cancer) or the WHO2010 grading system. This review aims to present those classification systems as well as list important genetic signatures in PanNETs.

Gross and microscopic findings

PanNETs are rare, small, well-demarcated lesions with the usual diameter less than 1 cm. Their slow growing makes the name "cancer in slow motion" and their silence in expression makes them metastasized before the patients becoming symptomatic. Microscopically, the neoplastic cells have a distinct neuroendocrine morphology characteristic by a granular amphophilic to eosinophilic cytoplasm and the typical coarsely clumped "salt and pepper" chromatin. The WHO2010 classification based on the mitotic rate and percentage of Ki67 positive cells for grading PanNETs (Table 1) [1] . Both grade 1 (<2 mitoses per 10 HPF; Ki-67 labeling index <3 %) and grade 2, (2–20 mitoses per 10 HPF; Ki-67 labeling index 3–20 %) constitutes of well-differentiated PanNETs. Grade 3 neuroendocrine tumor or neuroendocrine carcinoma (PanNEC) is considered when mitotic count is >20 mitoses per 10 HPF or Ki-67 index is >20 %. PanNECs with a very high proliferation rate (>50 %) can be expressed as small cell carcinoma or large cell carcinoma appearance. Those cells are tightly packed in nests or form diffuse irregular sheets. Necrosis is often seen. The ENETs[2] TNM grading, however, traditionally based on the size and the invasiveness of the tumors, but it is more preferable and is considered more accurate in grading the prognosis of PanNETs patients. (Table 2).

Table 1

2010 World Health Organization (WHO) classification of neuroendocrine tumors

Grade	Mitotic count (mitoses per 10 HPF)	Ki 67 index	Traditional nomenclature	WHO/ENETS nomenclature
Grade 1	< 2 mit/ 10 HPF	< 3%	Carcinoid, islet cell tumor	Neuroendocrine tumor, grade 1
Grade 2	2-20 mit/ 10 HPF	3-20%	(Atypical) Carcinoid, islet cell tumor	Neuroendocrine tumor, grade 2
Grade 3	> 20 mit/ 10 HPF	> 20%	Small cell carcinoma, large cell carcinoma	Neuroendocrine carcinoma (large cell or small cell)
Mixed adenoneuroendocrine carcinoma				
Hyperplastic and pre-neoplastic lesions				

Mit: motoses HPF: High power field

Table 2

2006 ENETS staging system for PanNETs

Stage	Tumour	Node	Metastasis	Stage specific 5 year survival	
I	T1	N0	M0	Stage I	97%
IIA	T2	N0	M0	Stage II	87%
IIB	T3	N0	M0	Stage III	73%
IIIA	T4	N0	M0	Stage IV	56%
IIIB	Any T	N1	M0		

IV	Any T	Any N	M1

T1: < 2cm but limited to the pancreas

T2: 2-4 cm but limited to the pancreas

T3: > 4cm but limited to the pancreas or invading the duodenum or common bile duct

T4: tumor invading adjacent structures or large vessels

N0: no regional lymph node metastases

N1: regional lymph node metastases

M0: no distant metastases

M1: distant metastases

Genetic signature: familial PanNET

90 % of PanNETs occur sporadically, but about 10% associate with the underlying genetic syndrome including multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau syndrome (VHL), neurofibromatosis type 1 (NF1), tuberous sclerosis complex (TSC) and the recently discovered glucagon cell adenomatosis (GCA)[3]. Therefore, deep studies of underlying genetic predisposition in those patients have provided important insight into the tumorigenesis of PanNETs. In these syndromes, the transformation of hyperplastic nodules over time finally creates the occurrence of frank neoplasms, and it is assumed that sporadic cases PanNETs also develop through a similar hyperplasia-neoplasia sequence.

Genetic signature: sporadic PanNET

Well-differentiated PanNETs (grade 1 and 2) in sporadic patients showed an average of only 16 mutations per tumors. 45% sporadic PanNETs patients express somatic mutations of the *MEN1* gene. 20% to 45% has loss of heterozygosity at the *MEN1* locus. In addition, PanNETs also harbored somatic inactivating mutations in *ATRX* or *DAXX* and had mutations in mTOR pathway genes. A mechanism of telomerase independent telomere maintenance to overcome cell death was found to correlate perfectly with loss of *ATRX* and *DAXX*, and the allelic loss of *PHLDA3* - a regulator of the mTOR pathway-was culprit in the deterioration of this pathway.

The genetic alterations are different between well-differentiated PanNETs (grades 1 and 2) and advanced PanNEC (grade 3). Although small and large cells PanNECs are genetically similar, they are distinct from PanNETs. *KRAS* activated mutation and *TP53* and *RB1* inactivated mutation were predominant in PanNECs, while they were not seen in 11 well-differentiated PanNETs. Furthermore, all PanNECs retained expression of *ATRX* and *DAXX*, while, as noted above, PanNETs showed loss of expression of *ATRX* and *DAXX* in 45 % of cases.

Epigenetic alterations

Unlike other tumors, epigenetic alterations in PanNETs have been mentioned in few studies. However, the change in methylation of tumor suppressor or oncogenic genes has been reported[4]. Hypomethylation in *LINE-1* was reported in 20 % of well-differentiated PanNETs, and strongly correlated with poor prognosis and high stage. Others found hypermethylation of the tumor suppressor gene *RASSF1A* in 75–80 % of

PanNETs with associated decreased protein expression of RASSF1A. RASSF1 family plays an important role in maintaining normal function of Wnt singaling pathway. In addition, the suppressing RASSF1A creates the escape of *miR-21*, which in turn, associates with both a high Ki67 proliferation index and metastasis to the liver.

MicroRNA

Studies of microRNA expression have suggested that *miR-193b* is a differential marker for PanNET in tissue and serum compared to normal. *MiR-103* and *miR-107* were also overexpressed and *miR-155* was downregulated in PanNET .

References

Mingyi Chen et al. (2012). *Molecular pathology of pancreatic neuroendocrine tumors*. J Gastrointest Oncol.

Yang M. et al. (2015). *TNM staging of pancreatic neuroendocrine tumors: an observational analysis and comparison by both AJCC and ENETS systems from 1 single institution*. Medicine.

LD Wood et al. (2015). *Genomic landscapes of pancreatic neoplasia. Journal of Pathology*.

Lauren P. Blair et al. (2012). *Epigenetic mechanisms in commonly occurring cancers. DNA Cell Bi*o.