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ORIGINAL ARTICLE

Pancreatic Intraepithelial Neoplasia (PanIN) as a Morphologic Marker of Pancreatobiliary Type of Ampullary Carcinoma

Kritika Krishnamurthy [1] · Vathany Sriganeshan [1,2]

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Abstract

The classification of ampullary adenocarcinoma into intestinal and pancreatobiliary sub-types has been found to be important in predicting prognosis and determining therapeutic strategy. Due to considerable inter-observer variability in sub-typing based solely on morphology, higher frequency of poorly differentiated cancers and low incidence of the disease, the histomorphologic classification of ampullary adenocarcinoma remains one of the grey zones in surgical pathology. Pan-IN is a well recognized precursor to pancreatic adenocarcinoma. Three studies have shown concurrent Pan-IN in patients with ampullary carcinoma, but their association with the two sub-types has not yet been reported. Fourteen cases of surgical resection for ampullary adenocarcinoma were retrieved from the archives. The cases were classified into two groups based on the presence or absence of concomitant Pan-IN. All the cases were stained for CK7, CK 20, Villin and CDX 2 and were classified as intestinal or pancreatobiliary types based on the staining pattern. All the 10 cases with Pan-IN stained negative for CDX2 and were classified as pancreatobiliary type ($p = 0.01$). Of the cases without Pan-IN, 3 were classified as intestinal sub-type based on morphology and CDX2 positivity and 1 was classified as pancreatobiliary type. Concomitant Pan-IN was present in 91% of pancreatobiliary type of ampullary adenocarcinoma. The grade of Pan-IN did not influence the grade or stage of the adenocarcinoma ($p > 0.05$). The co-occurrence of Pan-IN in a high percentage of the pancreatobiliary sub-type and its complete absence in the intestinal sub-type may serve as a strong differentiator between the two sub-types.

Keywords Pancreatic intraepithelial neoplasia (pan-IN) · Ampullary adenocarcinoma and its sub-types · Pancreatic adenocarcinoma · CDX2

Introduction

Ampullary carcinomas arise at the confluence of the pancreatobiliary and intestinal epithelium. These are rare neoplasms with an annual incidence of 4 to 6 cases per million and are 1.5 time more common in men as compared to women [1, 2]. They comprise less than 1% of all gastrointestinal neoplasms [3].

In 1994, Kimura reported two main histological sub-types of ampullary adenocarcinoma, intestinal and pancreatobiliary [4].

✉ Kritika Krishnamurthy
kritikakrishnamurthy@yahoo.com

[1] Arkadi M Rywlin Department of Pathology and Laboratory Medicine, Mount Sinai Medical Center, 4300 Alton road, Suite 2400, Miami Beach, FL 33140, USA

[2] FIU Herbert Wertheim college of Medicine, Miami, FL, USA

Intestinal type adenocarcinoma is the more prevalent sub-type of ampullary adenocarcinoma [5]. Multiple studies have shown the clinical and biological behavior of the intestinal type of ampullary adenocarcinoma to mirror their intestinal counterparts [6]. On the other hand, the pancreatobiliary type usually presents with more advanced disease than the intestinal type with higher frequency of perineural invasion [7]. Long-term survival after surgical treatment is significantly higher in patients with intestinal type than pancreatobiliary type [8].

Thus classification of ampullary adenocarcinomas is gaining importance in predicting the prognosis as well as determining the therapeutic strategy.

Isolated histological classification is hindered by inherent subjectivity and considerable inter-observer variability. Additionally, undifferentiated or poorly differentiated tumors cannot be classified based purely on tumor morphology [9].

Multiple immunohistochemical markers of intestinal and pancreatobiliary lineage have been tried both individually

and as panels to differentiate the two major histological types of ampullary carcinomas. Markers that have been extensively evaluated include CDX2, MUC1, MUC2, CK7, and CK20. However, only CDX2 and MUC1 were found to be of any relevance in the confirmation of the histological classification and prognostic evaluation [10, 11]. Other known markers of intestinal and pancreatobiliary lineage failed to conclusively corroborate the sub-type, creating ambiguity in cases with mixed histological types and poor differentiation [9].

Pancreatic intraepithelial neoplasia (Pan-IN) is a well recognized precursor to pancreatic adenocarcinoma. Multiple studies have shown concurrent Pan-IN in patients with ampullary carcinoma. In one study, high-grade intraepithelial neoplasia was present in the pancreatic ducts in 22% of resected tumors [12]. Another study found the incidence of Pan-IN to be similar between pancreatic adenocarcinomas and ampullary adenocarcinomas [13]. As yet no study has compared the association of Pan-IN among the different histological subtypes in ampullary carcinoma.

Materials and Methods

A retrospective review of our pathology archives was performed to identify all ampullary carcinomas resected between January 2010 and December 2018 at Mount Sinai Medical Center. Fourteen cases of segmental resection for ampullary adenocarcinoma were retreived from the archives.

The slides of all the fourteen cases were re-examined for presence of PanIN in random section of the pancreas. The PanIN was graded as per the three tier classification system as well as the newly proposed two tier classification system. The cases were classified into two groups based on the presence or absence of concomitant PanIN.

Immunohistochemical studies were performed in all cases from representative formalin-fixed paraffin-embedded blocks using the primary antibody CK7 (Agilent Pathology Solutions, Santa Clara, CA, USA; clone OV-TL 12/30), CK 20 (Agilent Pathology Solutions, Santa Clara, CA, USA; clone Ks20.8), CDX 2 (Biocare Medical, Pacheco, CA, USA; clone CDX2–88) and villin (Cell Marque Corporation, Rocklin, CA, USA; clone CWWB1), Ventana Benchmark automated slide stainer, and UltraView Universal Alkaline Phosphatase Red Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). Appropriate positive and negative controls were run simultaneously.

The stained slides were assessed for presence and absence of staining for CK7, CK20, CDX2 and Villin. The positively stained cases were further assessed for pattern and intensity of staining.

Statistical analysis was performed using SPSS version 22.0 software. Chi-square and Fischer exact test were performed. In view of the small number of cases, N Chi square tests were performed.

Results

A total of fourteen cases of ampullary carcinoma underwent resection at Mount Sinai Medical Center from January 2010 to December 2018. The age of the patients with ampullary carcinoma ranged from 59 to 85 years with a mean age of 72.2 ± 6.6 years. Of these, eight were men and 6 were women. At the time of resection, two patients had stage 1 disease, three had stage 2 disease, six had stage 3 disease and three had stage 4 disease. Six cases had lymph node metastasis. On the original reports, five of these cases had been diagnosed as ampullary adenocarcinoma, pancreatobiliary type and one as ampullary adenocarcinoma intestinal type. The reminder of the cases had been reported as ampullary adenocarcinoma, not otherwise specified.

On an average, pancreatic parenchyma was present in 5 sections per case. In cases classified as pancreatobilary type, pancreatic parenchyma was present in an average of 5 sections whereas in cases classified as intestinal type, an average of 4 sections where found to contain pancreatic parenchyma. This difference was, however, not statistically significant.

On re-examination of the pancreatic sections for PanIN, ten cases were found to have concomitant PanIN. In six cases the PanIN was classified as grade 1, three as grade 2 and one had grade 3.

On immunohisochemical staining with CK7, thirteen cases showed positive membranous staining in the tumor cells. The staining was patchy and focal in four of these cases. The tumor cells were positive for CK 20 in twelve cases. In seven caes the CK 20 was patchy and focal and four of theses cases showed weak staining. The tumor cells were positive for villin in eight cases. The CK 7, CK 20 and villin staining showed no correlation with presence of PanIN ($p > 0.05$).

Nuclear staining for CDX2 was negative in eleven cases and taken in conjunction with morphology, these were classified as pancreatobilary type. All the 10 cases with PanIN stained negative for CDX2 and were classified as pancreatobiliary type ($p = 0.01$). (Fig. 1).

Of the cases without PanIN, 3 were classified as intestinal subtype based on CDX2 positivity and 1 was classified as pancreatobiliary type. Concomitant PanIN was present in 91% of pancreatobiliary type of ampullary adenocarcinoma. The grade of PanIN did not influence the grade or stage of the adenocarcinoma ($p > 0.05$). Thee results are summarised in Table 1.

Discussion

The ampulla of Vater is the junction where three distinct epithelia coalesce: the duodenal, the pancreatic and the biliary. Thus carcinomas of the ampullary region can show differentiation towards any of the colliding epithelia contributing to the clinical heterogeniety of these neoplasms [14].

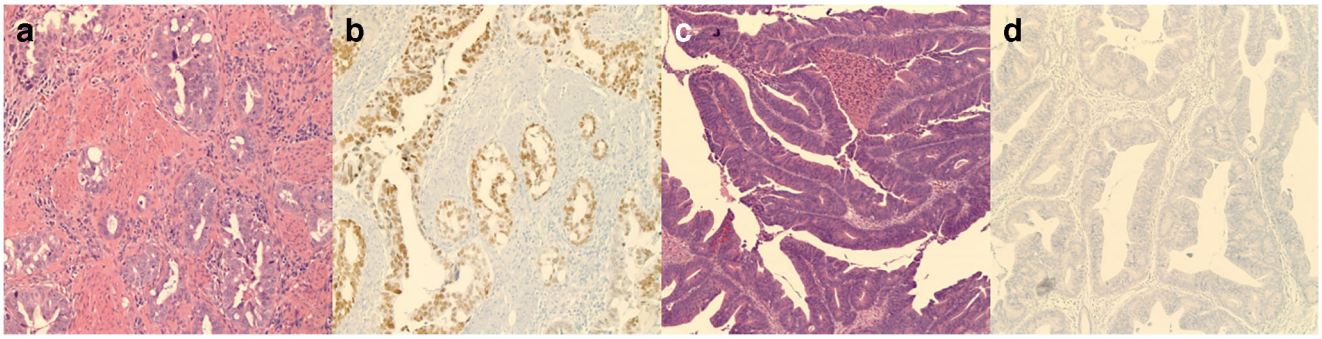

Fig. 1 Representative cases of (**a**) Intestinal subtype (20X), (**b**) Positive CDX 2 in intestinal type (20X), (**c**) Pancreatobiliary subtype (20X), (**d**) Negative CDX 2 in pancreatobiliary type

Adenocarcinomas comprise 90% of all ampullary malignancies, with other types such as adenosquamous and neuroendocrine accounting for the remainder [15, 16].

Two main histological sub-types of ampullary adenocarcinoma, the intestinal and the pancreatobiliary (pancreatobiliary) were first described by Kimura et al. in 1994 [5]. Despite the common anatomical location, the two sub-types differ not only in the histolomorphology of the tumor cells, but are associated with different premalignant lesions, have different cellular markers and gene expression,

differ in their mode of spread, and in their interaction with the extracellular matrix [9]. Thus, both subtypes have distinct molecular pathogeneses and prognosis. The intestinal type which are thought to evolve from the adenoma-carcinoma sequence have better long term survival rates as compared to the pancreatobiliary type, which are more frequently associated with perineural invasion and advanced disease [17, 18].

Thus histologic subtying of ampullary adenocarcioma appears to have significant prognostic and therapeutic implications. But due to the considerable inter-observer variablity in isolated morphology based subtyping, higher frequency of poorly differentiated cancers and low incidence of the disease, the histomorphologic classification of ampullary adenocarcinomas remains one of the grey zones in surgical pathology.

Due to different histogenesis, the two subtypes express different immunophenotypes and this difference can be exploited by immunohistochemical analysis. Potential markers to differentiate the intestinal and pancreatobiliary subtypes include cytokeratin 7, cytokeratin 20, MUC 1, MUC 2 and CDX2. Unfortunately, studies investigating these markers have shown that they are incapable of differentiating the two subtypes when used induvidually.19 Chang et al. evaluated five markers (CDX2, MUC1, MUC2, CK7, and CK20) as a combination panel for consistency in histological

Table 1 Summary fo results-

		Ampullary adenocarcinoma	
Age (mean ± Standard deviation)		72.2 ± 6 years	
Man: Women		8:6	
Stage	1	2	
	2	3	
	3	6	
	4	3	
Cases with lymphnode metastases		6/13	
CK 7 +		13/14	
CK 20 +		12/14	
Villin +		8/14	
		Pancreatobiliary type	Intestinal type
Pancreatic sections examined per case		5	4
*CDX2 +		0/11	3/3
*Concomitant PAN-IN		10/11	0/3
PAN-IN grade	1	6	NA
	2	3	
	3	1	

*P-value<0.05

subtyping. However, they found only CDX2 and MUC1 to be useful in the classification and prognostication in three independent patient cohorts. In this study, the pancreaticobiliary type of ampullary adenocarcinoma consistently stained negative for MUC1 and CDX2 and was associated with worse outcomes [11]. An independent study in 105 cases of ampullary adenocarcinoma by Ang et al. found that a four marker panel comprising of MUC1, CDX2, CK20 and MUC2 facilitated a dichotomous classification in 92% of the cases. Similar to the study by Chang et al., the intestinal subtype was defined by positive staining for CDX2 and MUC1 while the pancreatobiliary subtype was defined by MUC1 positivity in the absence of staining for CDX2 and MUC2. They found that immunophenotyping aided in the categorization of 75% of poorly differentiated adenocarcinomas and 69% of cases with mixed histologic features as either intestinal or pancreatobiliary subtype. However 8% of the cases could still not be classified due to morphological and immunohistochemical ambiguity [10].

More recently, Schueneman et al. proposed and tested a 92 gene panel assay for the classification of ampullary adenocarcinoma. They reported the sensitivity of the 92-gene assay and histomolecular classification to be similar for the intestinal subtype, however according to this study the 92-gene assay demonstrated higher sensitivity for the pancreatobiliary subtype [19]. In another study, 91 cases of ampullary adenocarcinoma were evaluated based on morphology, immunohistochemical panel including CK7, CK20, MUC1, MUC2 and CDX2 and a 50-gene panel mutational analysis. 20% of the cases remained ambiguous and could not be classified. This study concluded that mutational analysis and MUC5AC expression provide no additional value in the subtyping of ampullary adenocarcinomas [20].

Despite, the use of multiple immunohistochemical panels and molecular assay, the classification of ampullary adenocarcinomas remains dubious and inconclusive. In this scenario, the co-occurence of PanIN in a high percentage of the pancreatobiliary subtype and its complete absence in the intestinal subtype, as found in our study may serve as a strong differentiator between the two subtypes.

Multiple studies report similar molecular alterations in pancreatic adenocarcinoma and pancreatobiliary type of ampullary adenocarcinoma, hinting at a common evolution [12, 13]. Thus the concomitant presence of PanIN, may help rule out any uncertainity and reaffirm a pancreatobiliary subtyping in cases of ampullary adenocarcinomas. This is supported by the establishment of PanIN as a definite precursor of pancreatic adenocarcinoma and the identical molecular lanscape of pancreatic adenocarcinoma and pancreatobiliary type of ampullary adenocarcinoma.

Jeong et al. recently published a similar study where they found high-grade PanINs most commonly in pancreatic cancers and high-grade BilINs in distal bile duct cancers. According to their study high-grade PanINs were uncommon in ampullary or duodenal cancers. Thus they conluded that recognition of high-grade intraepithelial lesions can help identify the primary origin of periampullary cancers in ambiguous cases [21]. In our contemporaneous study we expanded upon the same concept in ampullary adenocarcinoma subtyping by including all grades of PanIN.

In the time of modern medicine, where morphology needs constant endorsement by immunohistochemistry and molecular modalities, the presence of concomitant PanIN may be used as a stand alone morphological marker for subtyping of ampullary adenocarcinomas. Not only would this hasten the diagnosis by eliminating additional testing and dubeity, but will also prove to be a more economically efficient alternative. However, in view of the small sample size in our study, the use of PanIN as a standalone marker needs to be further evaluated with a larger study population. Selective sequencing of the pancreatobilary type of ampullary adenocarcinoma and the concomittant PanIN using laser capture microdissection can further corroborate these findings by establishing a common genetic landscape of the two lesions.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

References

1. Albores-Saavedra J, Schwartz AM, Batich K, Henson DE (2009) Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. J Surg Oncol 100:598–605
2. Benhamiche AM, Jouve JL, Manfredi S, Prost P, Isambert N et al (2000) Cancer of the ampulla of Vater: results of a 20-year population-based study. Eur J Gastroenterol Hepatol 12:75–79
3. Winter JM, Cameron JL, Olino K, Herman JM, Jong MC et al (2010) Clinicopathologic analysis of ampullary neoplasms in 450 patients: implications for surgical strategy and long-term prognosis. J Gastrointest Surg 14:379–387
4. Kimura W, Futakawa N, Yamagata S, Wada Y, Kuroda A, Muto T, Esaki Y (1994) Different clinicopathologic findings in two histologic types of carcinoma of papilla of Vater. Jpn J Cancer Res 85(2): 161–166
5. Bronsert P, Kohler I, Werner M, Makowiec F, Kuesters S, Hoeppner J, Hopt UT, Keck T, Bausch D, Wellner UF (2013) Intestinal-type of differentiation predicts favourable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinomas of pancreatic, biliary, ampullary and duodenal origin. BMC Cancer 13:428
6. Ruemmele P, Dietmaier W, Terracciano L, Tornillo L, Bataille F et al (2009) Histopathologic features and microsatellite instability

of cancers of the papilla of Vater and their precursor lesions. Am J Surg Pathol 33:691–704
7. Albores-Saavedra J, Henson DE, Klimstra DS (2000) Malignant tumors of the ampulla. In:Tumors of the gallbladder extrahepatic bile ducts and ampulla of vater Armed Forces Institute of Pathology. Washington D.C, 259–316
8. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OPF, Gladhaug IP (2008) Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMCCancer 8:170
9. Rosa VDL, Peria FM, Brunaldi MO (2017) Carcinoma of ampulla of Vater: carcinogenesis and Immunophenotypic evaluation. J Clin Epigenet 3(2):25
10. Ang DC, Shia J, Tang LH (2014) The utility of immunohistochemistry in subtyping adenocarcinoma of the ampulla of Vater. Am J Surg Pathol 38:1371–1379
11. Chang DK, Jamieson NB, Johns AL (2013) Histomolecular phenotypes and outcome in adenocarcinoma of the ampulla of Vater. J Clin Oncol 31:1348–1356
12. Liu TH, Chen J, Zeng XJ (1983) Histogenesis of pancreatic head and ampullary region carcinoma. Chin Med J 96:167–174
13. Agoff SN, Crispin DA, Bronner MP, Dail DH, Hawes SE et al (2001) Neoplasms of the ampulla of Vater with concurrent pancreatic intraductal neoplasia: a histological and molecular study. Mod Pathol 14:139–143
14. Cattel RB, Pyrtek LJ (1949) An appraisal of pancreatoduodenal resection: a follow-up study of 61 cases. Ann Surg 129:840–848
15. Kimura W, Futakawa N, Zhao B (2004) Neoplastic diseases of the papilla of Vater. J Hepato-Biliary-Pancreat Surg 11:223–231
16. Zhou H, Schaefer N, Wolff M, Fischer H (2004) Carcinoma of the ampulla of Vater: comparative histologic/immunohistochemical classification and follow-up. Am J Surg Pathol 28:875–882
17. Fischer HP, Zhou H (2000) Pathologie der Papilla Vateri. In: Doerr W, Seifert G, Uehlinger E (eds) Spezielle Pathologische Anatomie: Pathologie der Leber und der Gallenwege, 2nd edn. Springer, Berlin Heidelberg New York Tokyo, pp 1219–1257
18. Sessa F, Furlan D, Zampatti C, Carnevali I, Franzi F, Capella C (2007) Prognostic factors for ampullary adenocarcinomas: tumor stage, tumor histology, tumor location, immunohistochemistry and microsatellite instability. Virchows Arch 451:649–657
19. Schueneman A, Wang H, Soifer HS, Schnabel CA, Wolff RA, Varadhachary GR, Overman MJ (2014) Molecular classification of ampullary adenocarcinoma: comparative prognostic performance of the 92-gene assay versus histomolecular analysis. J Clin Oncol 32(15):4141–4141
20. Perkins G, Svrcek M, Bouchet-Doumenq C, Voron T, Colussi O, Debove C, et al. (2019) Can we classify ampullary tumours better? Clinical, pathological and molecularfeatures. Results of an AGEO study. Br J Cancer [Epub ahead of print]
21. Jeong BK, Sung YN, Kim SJ, An S, Park H, Hwang HS, Kang HJ, Lee JH, Song KB, Kim KP, Hwang DW, Lee SS, Kim SC, Hong SM (2019) High-grade precursor lesions can be used as surrogate markers to identify the epicenter of periampullary carcinomas. Hum Pathol 84:92–104