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Original Article

Utility of HNF-1B and a panel of lineage-specific biomarkers to optimize the diagnosis of pancreatic ductal adenocarcinoma

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Abstract: Pancreatic ductal adenocarcinoma (PDAC) represents one of the most common cancers with dismal prognosis. Definitive diagnosis of PDAC remains challenging due to the lack of specific biomarkers. A transcription factor essential for pancreatic development named HNF-1B can be a potential biomarker for PDAC. However, HNF-1B was not entirely specific for PDAC and can be expressed in cancers of Müllerian tract, kidney, lung, bladder and prostate. To solve this issue, we investigated the expression of a panel of well-established lineage-specific biomarkers for non-pancreatic origins, including TTF1 and Napsin A for lung, RCC for kidney, ER and PR for breast, NKX3.1 for prostate, PAX8 for Müllerian tract, GATA3 for breast and bladder, and keratin CK7 and CK20 in 149 PDACs, using immunohistochemistry and tissue microarray. A two-tier scoring system for HNF-1B expression in tumor cells was used. Chi-square and Fisher's exact tests were performed using SAS software version 9.4 to test the association between HNF-1B expression and tumor morphology and differentiation. The results showed that PAX8 was focally positive in 6 cases (4.0%). GATA3 was focally positive in 5 cases (3.4%). Napsin A was all negative except for 1 case with focal weak staining. All other lineage-specific markers such as TTF1, RCC, ER, PR and NKX3.1 were completely negative in all PDACs. Consistent with our previous result, the majority of PDACs (88.6%) was positive for HNF-1B, including 78 cases (59.1%) with "strong" and 54 cases (40.9%) with "weak" staining pattern. There was no significant association between HNF-1B expression and cytoplasmic clearing morphology. Addition of keratins may further aid the diagnosis of PDAC since the majority of PDACs (84.6%) was CK7+/CK20-, only a minority of PDACs (11.4%) was CK7+/CK20+, 2.7% were CK-/CK20-, and 1.3% were CK7-/CK20+. In conclusion, HNF-1B can serve as a useful biomarker to aid the diagnosis of PDAC when combined with other lineage-specific biomarkers to exclude the other origins.

Keywords: HNF-1B, pancreatic ductal adenocarcinoma, tissue microarray, immunohistochemistry

Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of all pancreatic primary cancers. According to 2019 Cancer Statistics, PDAC is the tenth and ninth leading cancer type in males and females, respectively, and the incidence rate continues to increase [1]. Pancreatic cancer accounts for the fourth leading cancer-related death in both genders in the United States with the all-stage 5-year survival rate of 9% [2], which is the lowest among all cancers. With new emerging immunotherapy, neoadjuvant and adjuvant therapies, the 5-year overall survival rate is significantly improved to approximately 24% [3], even if the initial treatment was delayed [4]. PDAC is also one of the most common primaries in presentations of cancer from an unknown primary (CUP) [5]. CUP is associated with dismal prognosis with a median survival of 5-11 months after the initial diagnosis [6]. Due to the lack of specific serum biomarkers to diagnose PDAC, histomorphology and immunohistochemical studies become very informative to confirm the diagnosis, although this approach has been challenging.

Hepatocyte nuclear factor 1B (HNF-1B) is a nuclear transcription factor and is among the top enriched master regulators in the normal pancreas [7]. HNF-1B and its homodimer or heterodimer partner HNF-1A play an important role in early organogenesis including pancreatic development. Mutations of HNF-1B were associated with severe developmental abnormalities including pancreatic agenesis or hypoplasia, multicystic renal disease, abnormal hepatobiliary tract and abnormal Müllerian tract [8-10]. Although it is unclear if HNF-1B plays any role in carcinogenesis, it was highly expressed in certain types of cancers including ovarian clear cell carcinoma [11, 12], PDAC [13, 14], yolk sac tumor [15], and a few others [14, 16]. The decreased HNF-1B expression in poorly differentiated adenocarcinoma may suggest a tumor suppressor role of HNF-1B in PDAC [7, 13]. Recently our study demonstrated that HNF-1B was exclusively expressed in benign pancreatic ductal epithelium but not in the acini or islet cells, and high positivity of HNF-1B was observed in both primary and metastatic PDACs by immunohistochemistry (IHC) [14]. Except for in cancers mentioned above, HNF-1B was also infrequently expressed in lung adenocarcinoma, bladder urothelial carcinoma and prostate adenocarcinoma in our previous study [14]. Since PDAC and majority of its mimickers are typically positive for cytokeratin 7 (CK7), definitive diagnosis of PDAC can be extremely challenging for surgical pathologists in the absence of specific biomarkers. In this study, we intended to compare the expression of HNF-1B with a large panel of well-established lineage-specific biomarkers to aid the diagnosis of PDAC.

Materials and methods

Study materials

A total of 149 primary PDAC resection specimens were retrospectively retrieved from formalin fixed paraffin embedded (FFPE) blocks. This study was approved by Institution Review Board (IRB). All tumor slides were reviewed to document tumor histomorphology. The size of tumor and tumor grade were extracted from the electronic pathologic staging record and confirmed by reviewing representative slides. Two-millimeter core tissue microarrays (TMA) were constructed with duplication from one representative FFPE tumor block and adjacent non-neoplastic pancreas as internal control.

Immunohistochemistry (IHC)

TMA 5-μm sections of FFPE were stained by IHC with monoclonal antibodies against a panel of lineage-specific biomarkers including HNF-1B, ER, PR, RCC, GATA3, NKX3.1, TTF1, Napsin A, cytokeratin CK7 and CK20, and polyclonal antibody for PAX8. Briefly, deparaffinization and antigen retrieval were done using usual heat-based methods. All the conditions, reagents and detection were listed in **Table 1**. For HNF-1B, antigen retrieval was carried out with 1 mM EDTA (pH 8.0), followed by heating in a 770-Watt microwave oven for 14 minutes, cooled to room temperature, and rinsed in distilled water before staining procedure on the Dako Autostainer instrument (Agilent, Santa Clara, CA). Ventana UltraView DAB (Ventana Medical Systems) reagents were applied for detection, visualization and counterstaining.

An arbitrary 2-tier scoring system was used for HNF-1B expression: “strong” if the nuclear stain in any tumor cells was clearly visualized at 20 × magnifications; and “weak” if the nuclear stain in tumor cells was clearly visualized at 100 × magnifications.

Statistical analysis

For the first three statistical analyses, we performed the Pearson’s Chi-square test for all the data frequency tables, since at least 80% of the cells in the 2 × 3 tables had an expected value greater than 5. For the last analysis when the tables were sparse with small cell values, Fisher’s Exact test were conducted because of a large number of cells with expected count less than 5. For all these analyses, we used SAS software version 9.4 with Proc Freq.

Results

Morphological features of PDAC

Multiple variants of histomorphology of PDAC were observed including small glands, large ducts, clear cytoplasm, mucinous cells and undifferentiated cells with representative morphology showing in **Figure 1**. There was no significant difference of HNF-1B stain between

Table 1. Primary antibody information and IHC working conditions

Primary Antibody	Manufacturer	Clonality	Clone	Concentration	Control	Detection
HNF-1B	ThermoFisher cat# MA5-24605	Mouse monoclonal	CL0374	1:800	Pancreas	Ventana Ultraview
TTF1	Cell Marque cat# 343M-98	Mouse monoclonal	8G7G3/1	Ready to use	Thyroid	Ventana Ultraview
Napsin A	Ventana cat# 760-4867	Mouse monoclonal	MRQ-60	Ready to use	Lung	Ventana Ultraview
NKX3.1	Ventana cat# 760-5086	Rabbit monoclonal	EP356	Ready to use	Prostate cancer	Ventana Ultraview
GATA3	Cell Marque cat# 390M-18	Mouse monoclonal	L50-823	Ready to use	Breast ductal cancer	Ventana Ultraview
ER	Ventana cat# 790-4325	Rabbit monoclonal	SP1	Ready to use	Normal breast	Ventana Ultraview
PR	Ventana cat# 790-4296	Rabbit monoclonal	1E2	Ready to use	Normal breast	Ventana Ultraview
PAX8	Proteintech Group cat# 10336-1-AP	Rabbit polyclonal	---	1:100	Thyroid	Ventana Ultraview A&B Amp
RCC	Ventana cat# 760-4273	Mouse monoclonal	PN-15	Ready to use	RCC kidney	Ventana Ultraview
CK7	Ventana cat# 790-4462	Rabbit monoclonal	SP52	Ready to use	Tonsil	Ventana Ultraview
CK20	Ventana cat# 790-4431	Rabbit monoclonal	SP33	Ready to use	Colon	Ventana Ultraview

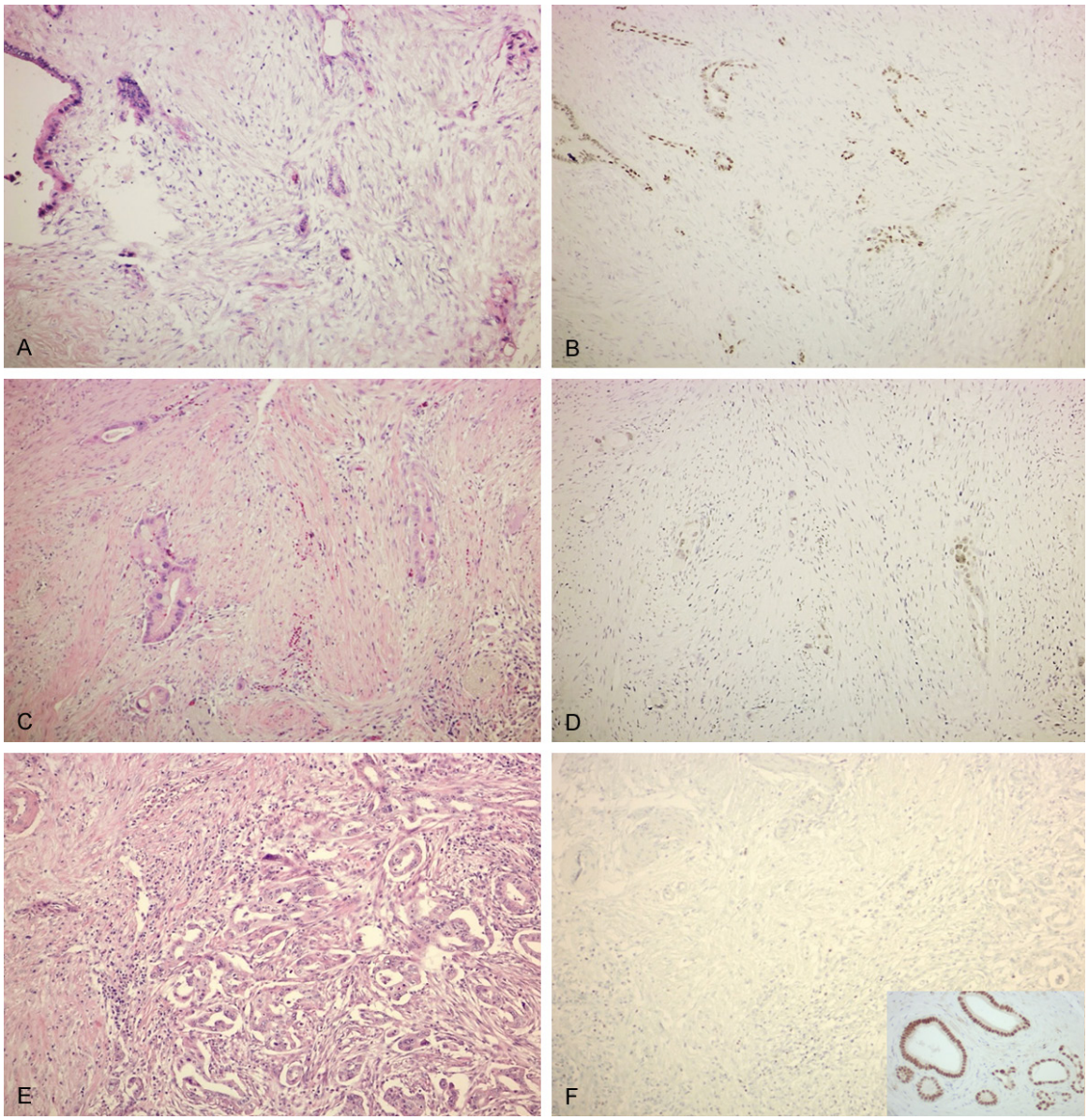

Figure 1. Variable HNF-1B nuclear expression in PDAC by IHC. (A) Representative case of PDAC on H&E stain and strongly positive for nuclear HNF-1B by IHC (B). (C) Representative case of PDAC on H&E stain and weakly positive for nuclear HNF-1B by IHC (D). (E) Representative case of PDAC on H&E stain and negative for HNF-1B by IHC (F). Insert in (F) showing HNF-1B positive control of adjacent non-neoplastic pancreatic ducts. Original magnifications: 100 ×.

the cases with clear cytoplasm and non-clear cytoplasm (P = 0.887). Among all 149 PDAC cases, 6 cases were well differentiated, 95 cases were moderately differentiated, and 48 cases were poorly differentiated.

HNF-1B expression in PDAC

HNF-1B was normally expressed in adjacent non-neoplastic pancreatic ductal epithelium with strong nuclear staining, which served as internal positive control (**Figure 1** insert). All the stains were nuclear and/or nuclear membranous with clean background, and no cytoplasmic or cytoplasmic membranous staining patterns were observed in this study with a monoclonal antibody. Of all the 149 PDAC cases, HNF-1B was expressed in a total of 132 cases (88.6%) with diffuse or patchy nuclear and/or nuclear membranous staining. Among

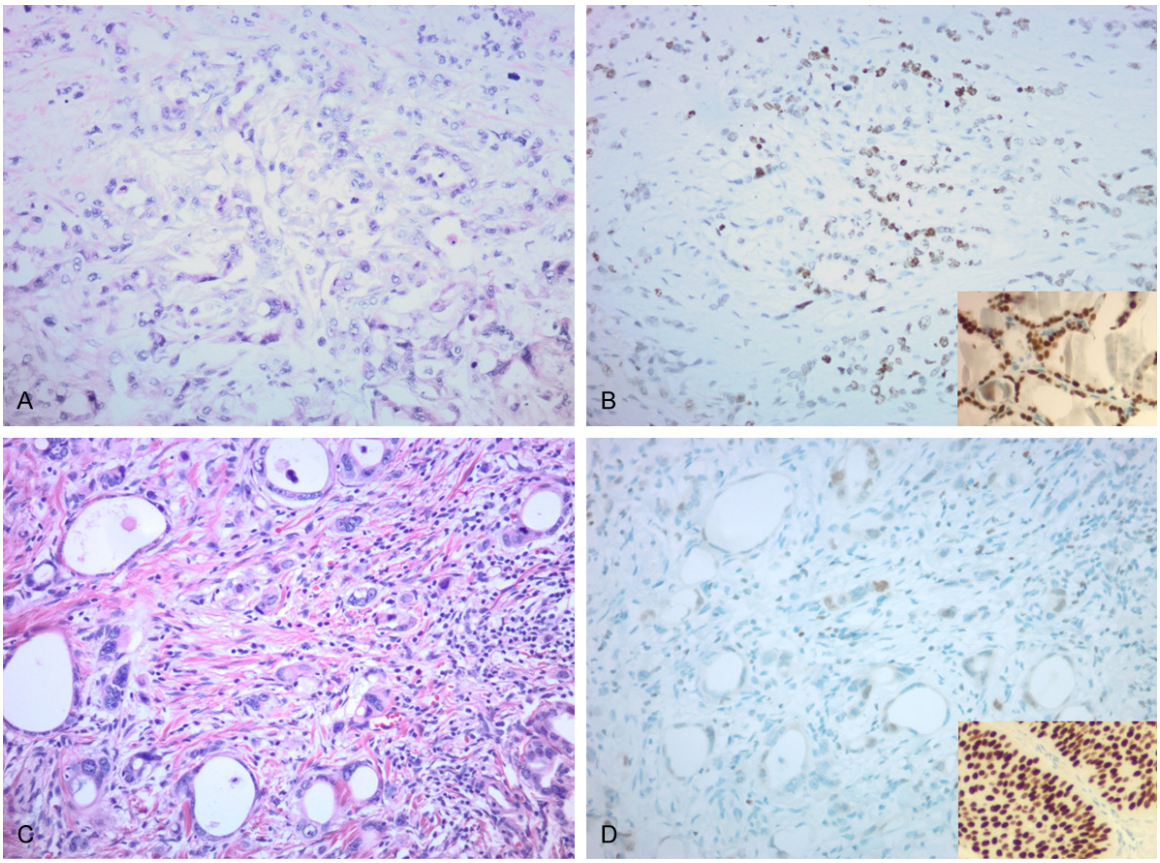

Figure 2. Representative case of PDAC on H&E stain (A) and showed weak positivity of PAX8 by IHC (B). Insert in (B) showing PAX8 positive control from benign thyroid tissue. Representative case of PDAC on H&E stain (C) and showed weak positivity of GATA3 by IHC (D). Insert in (D) showing positive control of GATA3 from benign urothelial epithelium. Original magnifications: 200 ×.

the 132 HNF-1B positive PDAC cases, 78 cases (59.1%) showed "strong" staining pattern (**Figure 1A**, **1B**), and 54 cases (40.9%) showed "weak" staining pattern (**Figure 1C**, **1D**). Within all 17 negative PDAC cases (**Figure 1E**, **1F**), 12 (70.6%) of them were poorly differentiated (grade 3), and 5 (29.4%) of them were moderately differentiated (grade 2). Fisher's exact test showed that there was statistically significant difference of HNF-1B expression among the tumor differentiation of grade 3 and combined grade 1 and 2 ($P = 0.004$).

CK7 and CK20 expression pattern in PDAC

The majority of PDACs (126/149 cases, 84.6%) were positive for keratin CK7 and negative for CK20 with few exceptions in this cohort: 17/149 cases (11.4%) were CK7 and CK20 both positive, 4/149 cases (2.7%) were both CK7 and CK20 negative, and 2/149 cases (1.3%) were CK7 negative and CK20 positive.

Focal expression of PAX8, GATA3 and Napsin A in PDAC

Of all the 149 PDAC cases, PAX8 was focally expressed in 6 cases (4.0%) with nuclear staining (**Figure 2A**, **2B**). Five of them were poorly differentiated and one of them was moderately differentiated. Among the 5 PAX8 focal positive poorly differentiated cases, two of which were HNF-1B negative.

GATA3 was focally expressed in 5 cases (3.4%) with nuclear staining (**Figure 2C**, **2D**). Three of them were moderately differentiated and two of them were poorly differentiated. Among two GATA3 focal positive poorly differentiated cases, one showed strong positive HNF-1B staining and the other one showed negative HNF-1B staining. Napsin A was all negative except for 1 case (0.7%) with focal weak staining (data not shown), while the HNF-1B in this case was strongly positive. PAX8 positive con-

Table 2. Expected reactivity of biomarkers in PDAC

Biomarkers	Reactivity in PDAC
CK7	+
CK20	-
HNF-1B	+
CDX-2*	-
TTF1	-
Napsin A	-
GATA3	-
ER	-
PR	-
PAX8	-
NKX3.1	-
RCC	-

*Note: CDX2 was based on our previous study result [14].

trol from benign thyroid tissue and GATA3 positive control from bladder were stained appropriately (**Figure 2B** and **2D** inserts, respectively).

TTF1, RCC, ER, PR and NKX3.1 expression in PDAC

The remaining biomarkers including TTF1, RCC, ER, PR and NKX3.1 were completely negative in all PDACs. PR stained normal endocrine cells in adjacent pancreatic islet cells; however, PR was completely negative in all PDACs. In other words, well-established lineage specific biomarkers for the breast, lung, prostate, kidney and Müllerian tract origin were largely negative in PDAC (**Table 2**). All positive controls were stained appropriately for each marker.

Discussion

PDAC is one of the most common cancers frequently presented with metastasis, and definitive diagnosis of PDAC in surgical pathology practice remains challenging due to the lack of specific biomarkers. We hypothesized that transcription factor HNF-1B essential for normal pancreas development may serve as a potential diagnostic marker for PDAC. In our previous study [14], a polyclonal antibody against HNF-1B showed high sensitivity but suboptimal specificity for the diagnosis of PDAC. To further determine the utility of HNF-1B as a biomarker for PDAC, exclusion of non-pancreatic origins using a panel of lineage-specific markers for the Müllerian tract, kidney, lung, bladder and prostate is essential [11, 17-20]. Consistent with our previous results [14], HNF-1B was highly expressed in PDACs with a positive rate of 88.6% using a monoclonal antibody against HNF-1B in current study. Importantly, most other lineage-specific biomarkers were not expressed in PDAC, except for PAX8 and GATA3 rarely showing focal positivity in PDAC. This result greatly increased the confidence that HNF-1B can serve as the biomarker for PDAC when other lineage-specific markers are negative. To our knowledge, this is the first investigation of a large panel of lineage-specific biomarkers in PDACs.

The role of HNF-1B in PDAC carcinogenesis or progression is not entirely clear. In this cohort, the majority (70.1%) of HNF-1B negative PDACs was poorly differentiated carcinomas, whereas the minority (29.4%) of HNF-1B negative cases was moderately differentiated carcinomas. HNF-1B negativity in tumor cells was significantly associated with tumor differentiation grade 3 compared with grade 1 and 2. In other words, HNF-1B was more likely to be positive in well to moderately differentiated PDACs and can be attenuated or lost in poorly differentiated cancers. Interestingly, a recent study by Janky and colleagues showed a gradual loss of nuclear HNF-1B expression from well differentiation towards moderate and poor differentiation of PDAC [7], which is consistent with our findings. Other studies have shown that HNF-1B seems to play an essential role in the pathogenesis of both prostatic and ovarian cancers [11, 19], to some extent reflecting its developmental role in these organs. In addition, in vitro studies suggested that HNF-1B may play a tumor suppressor role and suppress epithelial-to-mesenchymal transition (EMT), a well-characterized mechanism for cancer progression and metastasis [21]. Overexpression of HNF-1B in a prostatic cancer cell line triggered mesenchymal-to-epithelial morphological transition [21]. These findings suggest that loss of HNF-1B expression in cancer cells including PDAC might contribute to cancer progression. Although other report has shown an association of strong staining of HNF-1B with clear cytoplasmic variant of PDAC and poor prognosis [13], there was no statistical signifi-

cance between the HNF-1B expression and histomorphology in this cohort.

Conclusion

IHC is a feasible and cost-effective test routinely performed in surgical pathology labs. HNF-1B can be a useful biomarker to aid the diagnosis of PDAC when other lineage-specific biomarkers are negative to exclude the Müllerian tract, kidney, lung, prostate, and bladder origins by IHC. Combined with our previous study, a minimal panel including CK7, CK20, HNF-1B and CDX2 is suggested in the workup of PDAC, and the most common predicted immunophenotype would be CK7+, CK20-, HNF-1B+ and CDX2- in PDAC.

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Disclosure of conflict of interest

None.

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References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.

[3] Fong ZV, Chang DC, Hur C, Jin G, Tramontano A, Sell NM, Warshaw AL, Fernandez-Del Castillo C, Ferrone CR, Lillemoe KD and Qadan M. Variation in long-term oncologic outcomes by type of cancer center accreditation: an analysis of a SEER-medicare population with pancreatic cancer. Am J Surg 2020; 220: 29-34.

[4] Turner MC, Masoud SJ, Cerullo M, Adam MA, Shah KN, Blazer DG 3rd, Abbruzzese JL and Zani S. Improved overall survival is still observed in patients receiving delayed adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic adenocarcinoma. HPB (Oxford) 2020; S1365-182X(20)30087-3.

[5] Pentheroudakis G, Golfinopoulos V and Pavlidis N. Switching benchmarks in cancer of unknown primary: from autopsy to microarray. Eur J Cancer 2007; 43: 2026-2036.

[6] van de Wouw AJ, Janssen-Heijnen ML, Coebergh JW and Hillen HF. Epidemiology of unknown primary tumours; incidence and population-based survival of 1285 patients in Southeast Netherlands, 1984-1992. Eur J Cancer 2002; 38: 409-413.

[7] Janky R, Binda MM, Allemeersch J, Van den Broeck A, Govaere O, Swinnen JV, Roskams T, Aerts S and Topal B. Prognostic relevance of molecular subtypes and master regulators in pancreatic ductal adenocarcinoma. BMC Cancer 2016; 16: 632.

[8] El-Khairi R and Vallier L. The role of hepatocyte nuclear factor 1beta in disease and development. Diabetes Obes Metab 2016; 18 Suppl 1: 23-32.

[9] Naqvi AAT, Hasan GM and Hassan MI. Investigating the role of transcription factors of pancreas development in pancreatic cancer. Pancreatology 2018; 18: 184-190.

[10] Haldorsen IS, Vesterhus M, Raeder H, Jensen DK, Sovik O, Molven A and Njolstad PR. Lack of pancreatic body and tail in HNF1B mutation carriers. Diabet Med 2008; 25: 782-787.

[11] Tsuchiya A, Sakamoto M, Yasuda J, Chuma M, Ohta T, Ohki M, Yasugi T, Taketani Y and Hirohashi S. Expression profiling in ovarian clear cell carcinoma: identification of hepatocyte nuclear factor-1 beta as a molecular marker and a possible molecular target for therapy of ovarian clear cell carcinoma. Am J Pathol 2003; 163: 2503-2512.

[12] Huang W, Cheng X, Ji J, Zhang J and Li Q. The application value of HNF-1beta transcription factor in the diagnosis of ovarian clear cell carcinoma. Int J Gynecol Pathol 2016; 35: 66-71.

[13] Kim L, Liao J, Zhang M, Talamonti M, Bentrem D, Rao S and Yang GY. Clear cell carcinoma of the pancreas: histopathologic features and a unique biomarker: hepatocyte nuclear factor-1beta. Mod Pathol 2008; 21: 1075-1083.

[14] Yang MX, Coates RF, Ambaye A, Gardner JA, Zubarick R, Gao Y, Skelly J, Liu JG and Mino-Kenudson M. Investigation of HNF-1B as a diagnostic biomarker for pancreatic ductal adenocarcinoma. Biomark Res 2018; 6: 25.

[15] Rougemont AL and Tille JC. Role of HNF1beta in the differential diagnosis of yolk sac tumor from other germ cell tumors. Hum Pathol 2018; 81: 26-36.

[16] Yu DD, Guo SW, Jing YY, Dong YL and Wei LX. A review on hepatocyte nuclear factor-1beta and tumor. Cell Biosci 2015; 5: 58.

[17] Conner JR, Hirsch MS and Jo VY. HNF1beta and S100A1 are useful biomarkers for distin-

guishing renal oncocytoma and chromophobe renal cell carcinoma in FNA and core needle biopsies. Cancer Cytopathol 2015; 123: 298-305.

[18] Davidson B. Hepatocyte nuclear factor-1beta is not a specific marker of clear cell carcinoma in serous effusions. Cancer Cytopathol 2014; 122: 153-158.

[19] Elliott KS, Zeggini E, McCarthy MI, Gudmundsson J, Sulem P, Stacey SN, Thorlacius S, Amundadottir L, Grönberg H, Xu J, Gaborieau V, Eeles RA, Neal DE, Donovan JL, Hamdy FC, Muir K, Hwang SJ, Spitz MR, Zanke B, Carvajal-Carmona L, Brown KM; Australian Melanoma Family Study Investigators, Hayward NK, Macgregor S, Tomlinson IP, Lemire M, Amos CI, Murabito JM, Isaacs WB, Easton DF, Brennan P; PanScan Consortium, Barkardottir RB, Gudbjartsson DF, Rafnar T, Hunter DJ, Chanock SJ, Stefansson K and Ioannidis JP. Evaluation of association of HNF1B variants with diverse cancers: collaborative analysis of data from 19 genome-wide association studies. PLoS One 2010; 5: e10858.

[20] Hanley KZ, Cohen C and Osunkoya AO. Hepatocyte nuclear factor-1beta expression in clear cell renal cell carcinoma and urothelial carcinoma with clear cell features: a potential diagnostic pitfall. Appl Immunohistochem Mol Morphol 2017; 25: 134-138.

[21] Ross-Adams H, Ball S, Lawrenson K, Halim S, Russell R, Wells C, Strand SH, Orntoft TF, Larson M, Armasu S, Massie CE, Asim M, Mortensen MM, Borre M, Woodfine K, Warren AY, Lamb AD, Kay J, Whitaker H, Ramos-Montoya A, Murrell A, Sørensen KD, Fridley BL, Goode EL, Gayther SA, Masters J, Neal DE and Mills IG. HNF1B variants associate with promoter methylation and regulate gene networks activated in prostate and ovarian cancer. Oncotarget 2016; 7: 74734-74746.