Virchows Archiv (2021) 478:1215–1219
https://doi.org/10.1007/s00428-020-02942-1

BRIEF REPORT

Evidence of a common cell origin in a case of pancreatic mixed intraductal papillary mucinous neoplasm–neuroendocrine tumor

Marco Schiavo Lena[1] · Maria Giulia Cangi[1] · Lorenza Pecciarini[1] · Ilaria Francaviglia[1] · Greta Grassini[1] · Renaud Maire[2] · Stefano Partelli[3] · Massimo Falconi[3] · Aurel Perren[2] · Claudio Doglioni[1]

Received: 13 August 2020 / Revised: 18 September 2020 / Accepted: 27 September 2020 / Published online: 1 October 2020

Abstract

Recently, the term mixed neuroendocrine non-neuroendocrine neoplasms (MiNEN) has been proposed as an umbrella definition covering different possible combinations of mixed neuroendocrine-exocrine neoplasms. Among these, the adenoma plus neuroendocrine tumor (NET) combination is among the rarest and not formally recognized by the 2019 WHO Classification. In this setting, the debate between either collision tumors or true mixed neoplasms is still unsolved. In this report, a pancreatic intraductal papillary mucinous neoplasm (IPMN) plus a NET is described, and the molecular investigations showed the presence in both populations of the same KRAS, GNAS, and CDKN2A mutations and the amplification of the CCND1 gene. These data prove clonality and support a common origin of both components, therefore confirming the true mixed nature. For this reason, mixed neuroendocrine-exocrine neoplasms, in which the exocrine component is represented by a glandular precursor lesion (adenoma/IPMN) only, should be included into the MiNEN family.

Keywords Pancreatic neuroendocrine tumor · Intraductal papillary mucinous neoplasm · Mixed neuroendocrine non-neuroendocrine neoplasms · KRAS and GNAS mutation · CDKN2A mutation · Cyclin D1 amplification

Introduction

The classification of mixed neuroendocrine-exocrine neoplasms changed recently: the 2019 WHO Classification of Digestive System Tumors [1] extends the concept of mixed neuroendocrine non-neuroendocrine neoplasms (MiNEN) to all gastro-entero-pancreatic sites. The most common combinations include high-grade malignant components, which correspond to the old term mixed adenoneuroendocrine carcinoma (MANEC) of the 2010 WHO Classification of Tumors of the Digestive System [2].

However, there are also "low-grade" combinations, i.e., well-differentiated neuroendocrine tumor plus adenoma. This later combination is very rare, and a limited number of cases have been described in the GI tract [3]. In the pancreas, small case series reported neuroendocrine tumor/microadenoma with intraductal papillary mucinous neoplasms [4].

While for the high-grade combination, the origin of both components from a common precursor has been proven using molecular techniques [5]; it is still unclear whether the rarer low-grade counterpart represents either collision tumors or true clonal mixed neoplasm.

Case report

A 56-year-old man underwent pancreatoduodenectomy for a 30 mm intraductal papillary mucinous neoplasm (IPMN) of the pancreatic head, intestinal type, with high-grade dysplasia [1], of the branch ducts with extension to the main duct. Within the IPMN, a morphologically defined second component of a neuroendocrine tumor (NET) is recognized amidst the mucinous papillae (Fig. 1). This NET was 7 mm in diameter with a Ki-67 proliferative index up to 8–10% (G2).

✉ Marco Schiavo Lena
schiavolena.marco@hsr.it

[1] Pathology Unit, Pancreas Translational and Clinical Research Center, ENETS Center of Excellence, IRCCS San Raffaele Scientific Institute, via Olgettina 60, 20132 Milan, Italy

[2] Institute of Pathology, University of Bern, Bern, Switzerland

[3] Pancreatic Surgery Unit, Pancreas Translational and Clinical Research Center, ENETS Center of Excellence, IRCCS San Raffaele Scientific Institute, Milan, Italy

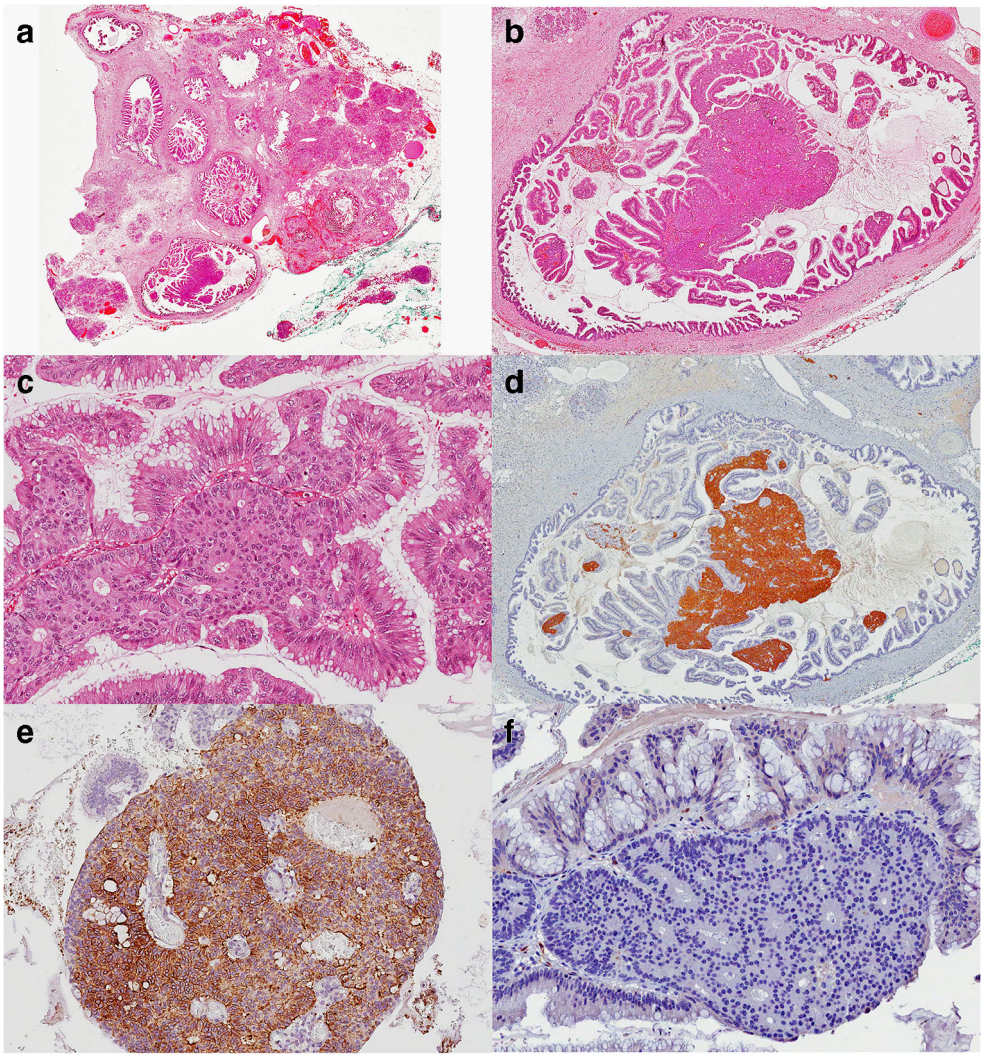

Fig. 1 Panoramic view of a section (**a**) showing a pancreatic intraductal papillary mucinous neoplasm (IPMN) of the peripheral ducts. At the bottom, a solid area is visible inside a dilated duct and amidst with the fronds of mucinous cells. At higher magnification (**b**, **c**). a double population is observed, mucinous-type at the external surface, and neuroendocrine-type in the deeper part of the neoplasm. The neuroendocrine component was intensely positive for immunohistochemical staining for synaptophysin (**d**) and SSTR2A (**e**). P16 resulted completely negative in both mucinous and neuroendocrine population (**f**)

The neuroendocrine nature was confirmed by widespread positivity to synaptophysin and chromogranin A, while the mucinous cells stained completely negative for these markers.

SSTR2A stained the membrane of 100% of NET cells. DAXX and ATRX nuclear staining was preserved. Glucagon, insulin, serotonin, and somatostatin resulted completely negative. Menin nuclear expression was retained in both populations; p16 nuclear staining instead was completely negative in both components.

Cyclin D1 stained intensely the nuclei of both components; the IPMN's positivity was stronger in the mucinous cells near the NET.

FISH analysis was performed to evaluate CCND1 gene copy number status and confirm amplification: high-level CCND1 gene amplification (> 10, clusters) is observed in both the IPMN and NET components (Fig. 2).

The molecular analysis carried out on the IPMN component showed the presence of KRAS mutation p.Gly12Asp (c.35G > A), GNAS mutation p.Arg201His (c.602G > A), CDKN2A mutation p.Thy44ter (c.131_132insA), and CCND1 amplification (copy number 28). The same molecular alterations are identified in the neuroendocrine component (Fig. 3), isolated by laser capture microdissection at the Institute für Pathologie of Bern, Switzerland, before DNA extraction.

Lymph nodes retrieved from the specimen were free of tumor (0/28). The post-operative course of the patient was uneventful. Currently, at 27 months of regular follow-up, he is healthy and disease-free.

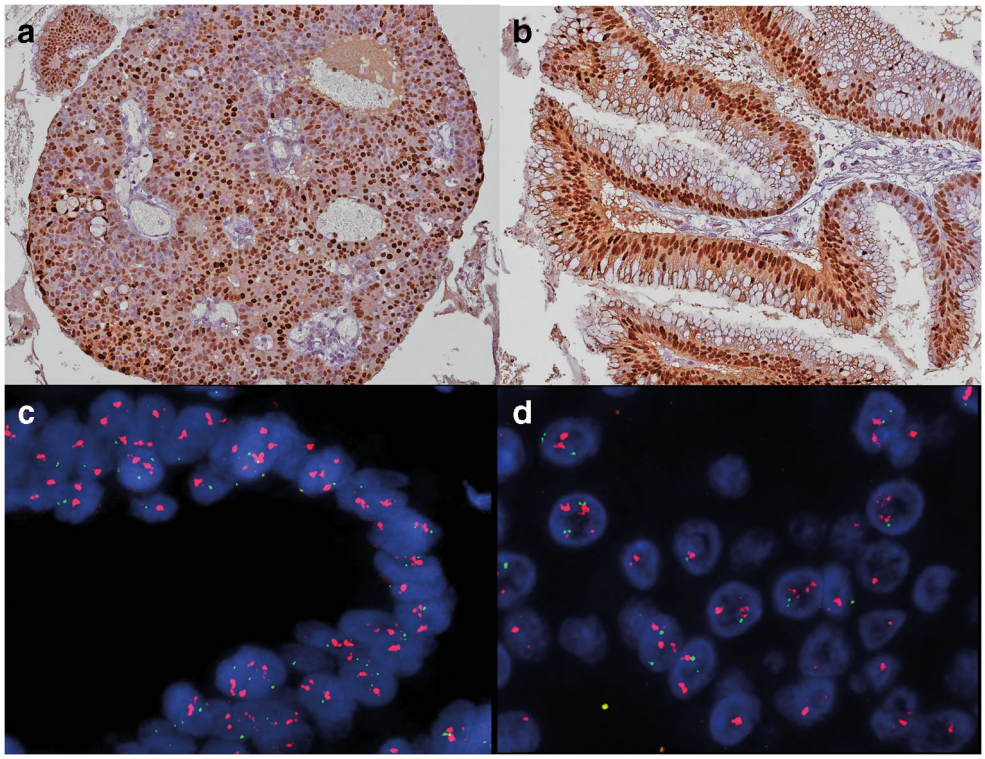

Fig. 2 Cyclin D1 nuclear expression was documented in both neuroendocrine cells (**a**) and mucinous cells of the intraductal papillary mucinous neoplasm (IPMN) (**b**). Likewise, CCND1 gene high amplification was found in both the neuroendocrine component (**c**) and the exocrine/IPMN component (**d**)

Material and methods

Serial 5-mm thick paraffin sections were collected on charged slides and processed using an automated immunostainer (BenchMark ULTRA; Ventana Medical System, Tucson, AZ). Antibodies against the following antigens were used: ATRX (polyclonal, Sigma Aldrich), chromogranin A (cl. LK2H10, Ventana), cyclin D1 (cl. SP4-R, Ventana), DAXX (polyclonal, Sigma Aldrich), glucagon (polyclonal, Ventana), insulin (polyclonal, Ventana), Ki67 (cl. 30–9, Ventana), Menin (polyclonal, Epitomics), p16 (cl. E6H4, Ventana), serotonin (polyclonal, Novocastra), somatostatin (polyclonal, Ventana), SSTR2A (cl. RM- UMB1, Epitomics), and synaptophysin (cl. SP11, Ventana) on whole sections.

Laser capture microdissection of cells for molecular analyses (Institute für Pathologie, Bern)

Five 7-μm-thick sections were cut on Leica PEN-Membrane 2.0-μm slides (Leica Microsystems GmbH, Germany) and stained in 1% Cresyl violet acetate solution for laser capture microdissection (LCM) using a Zeiss PALM MicroBeam 4.2 laser microdissection system (Carl Zeiss Microscopy GmbH). Cells, of a total surface of 3×10^6 uM^2, were dissected and catapulted directly into a Zeiss Adhesive Cap 500 opaque tube (Carl Zeiss Microscopy GmbH) and were lysed in 20 μl of ATL lysis buffer, containing proteinase k, overnight at 56 °C. Genomic DNA was then extracted using the QIAamp DNA micro kit (Qiagen, Milan, Italy) according to the manufacturer's instructions.

DNA extraction and sequencing methods

Targeted NGS was performed for molecular characterization of the two tumor components using the Oncomine Comprehensive DNA Assay v.3M (OCAv3, Thermo Fisher Scientific), according to manufacturer's protocols: genomic DNA from FFPE tumor tissue sections was isolated by automated extraction using the Maxwell® RSC instrument (Promega Italia S.r.l., Milano, Italy) following the manufacturer's protocols. Quantity of isolated DNA was assessed by Qubit 3.0 fluorometer (Thermo Fisher Scientific); sequencing was carried on S5 Ion Torrent (Thermo Fisher), and data analysis was performed by Ion Reporter™ Server hosting informatic tools (Ion Reporter™ Software v5.12) for variant analysis, filtering, and annotations.

FISH method

CCND1 gene copy-number (CN) status was assessed by fluorescence in situ hybridization (FISH) on formalin-fixed, paraffin-embedded tissue 4-μm tumor tissue sections using a

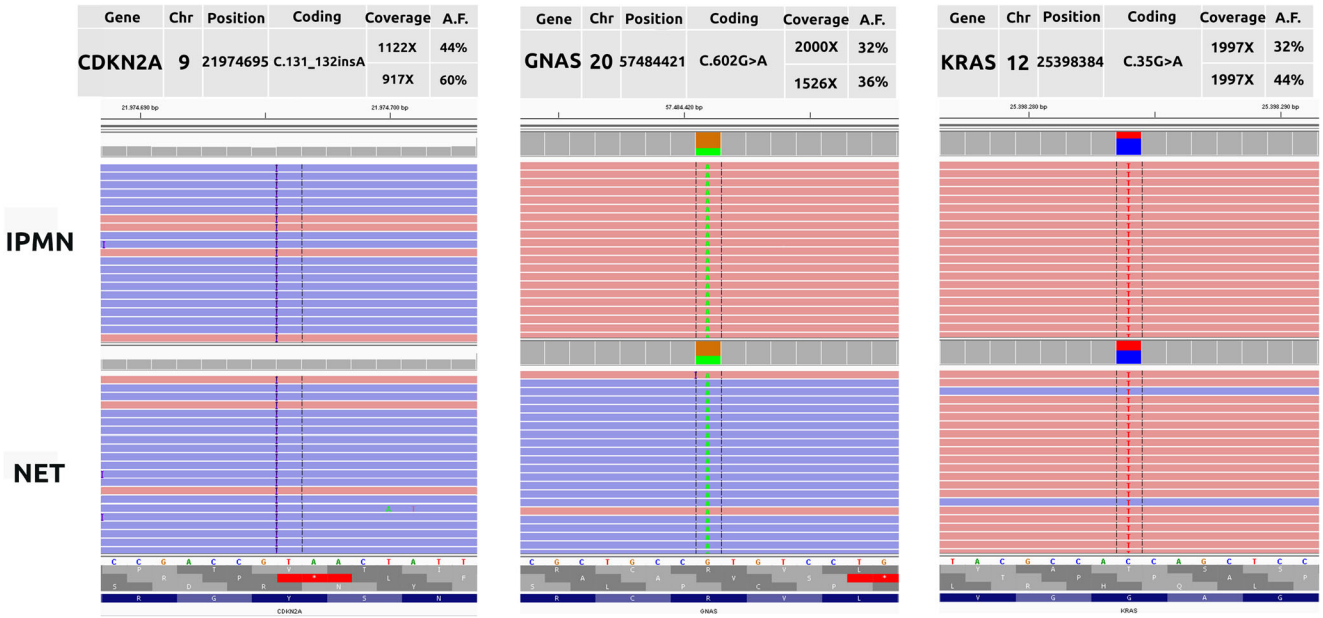

Fig. 3 CDKN2A, GNAS, and KRAS gene alterations were found in both the intraductal papillary mucinous neoplasm (IPMN) component (first row) and the neuroendocrine component (second row). Next-generation sequencing data, visualized with the integrative genomic viewer (IGV) software, revealed identical genetic alteration in the two populations (forward and reverse reads are shown in red and blue, respectively), with different allelic frequencies

dual-color FISH probe set (CCND1/CEN 11 Dual Color Probe, Cytocell, UK), following manufacturer's protocols. Slides were analyzed using Nikon 90i fluorescence microscope (Nikon Instruments SPA, Italy), and images were captured by Genikon software (Nikon). CCND1 CN per nucleus was counted in at least 100 tumor cells; high-level gene amplification was defined as more than 10 copies per nucleus or high copy clusters in > 50% of the cells; low-level amplification was defined as 6–10 copies in > 50% of cells; and 1–5 copies defined non-amplification.

Discussion

The 2017 WHO Classification of Tumors of Endocrine Organs [6], referring to the pancreas, introduced the term MiNEN. This term embraces a greater number of combinations of tumors than the previous MANEC, restricted to "adenocarcinoma" and "neuroendocrine carcinoma," of the 2010 WHO Classification [2]. Among them, the combination of well-differentiated neuroendocrine tumor and adenoma is one of the rarest.

In 2018 La Rosa et al. published [3] a series of such gastrointestinal tumors and proposed the term mixed adenoma well-differentiated tumor (MANET) to define them.

Here, we report a patient with a mixed tumor composed of a pancreatic IPMN and NET G2.

Various associations between IPMN and neuroendocrine tumor have been described in the literature. However, as recently highlighted [4], there is no clear separation between coexistence of incidental pancreatic microadenoma/small NET with exocrine tumor and true mixed IPMN-neuroendocrine tumor; the existence of the latter has not been proven molecularly.

In most of the cases described, IPMN and NET were topographically separated lesions that merely coexist in the same pancreas, and they probably represent two separate tumors [4]. To the best of our knowledge, only 3 cases of a truly mixed IPMN plus NET have been reported [7–9] (case number 5 described by Marrache [7]). In two of them, the neuroendocrine component was less than 5 mm in size.

La Rosa in his study on the MANET of the GI tract [3] hypothesized a common origin for both the neuroendocrine and non-neuroendocrine components, because of an intimate connection between the two populations observed on morphological ground. However, a common origin could not be proven molecularly, since both components were negative for the studied mutations (KRAS, BRAF, PIK3CA, and microsatellite instability).

Such an intricate morphological connection is rare between IPMN and NET, leaving the question of collision tumor versus true mixed tumors unanswered.

In the present case, we were able to prove clonality based on shared molecular abnormalities in both IPMN and NET. The predominant population was represented by the IPMN or the exocrine component. In fact, both populations shared two molecular alterations (KRAS and GNAS mutations) that are common in IPMNs, especially with intestinal phenotype and thought to be the earliest driver gene alterations of these neoplasms [1],

suggesting that the NET originated from IPMN. Conversely, these mutations are usually not detected in pancreatic NET [10].

Loss of p16 expression, through CDKN2A gene mutation, 9p21 deletion or promoter hypermethylation, has been described in IPMNs, and this event typically occurs after KRAS mutation and is more prevalent in high-grade dysplasia and with intestinal phenotype [11].

Alterations of the CDKN2A gene have also been described in PanNETs [1, 10]; however, when present they are associated with larger size, high grade, stage and the presence of distant metastases [12]. In the current case, the NET component was 7 mm only, and it seems more likely that the mutation of CDKN2A has been developed originally in the exocrine component.

In addition, our in situ analysis proved CCND1 gene amplification and immunohistochemical overexpression of its product, cyclin D1, in both cellular components.

While not expressed by normal pancreatic islets, immunohistochemical stain of cyclin D1 has been documented in pancreatic NETs without any amplification or rearrangement of its gene locus been detected [13], while the corresponding chromosomal region (11q13) is commonly deleted in pancreatic NETs [10].

For the exocrine counterpart, cyclin D1 immunohistochemical stain has been well-documented in pancreatic ductal adenocarcinoma (PDAC) and its putative precursor lesions, both pancreatic intraepithelial neoplasia (PanIN) and IPMNs [14]. Moreover, CCND1 amplification was found both in PDAC and IPMNs, intestinal subtype, with high-grade dysplasia [15].

In the 2019 WHO Classification [1], MANET was not recognized as an entity, since mixed NET and adenoma were not included in the MiNEN category, perhaps do to the lack of prove of clonality. The case presented here demonstrated that clonal mixed NET-adenoma/IPMN does really exist, and in the future, this combination should be included at the lower end of the MiNEN family.

Acknowledgments The authors want to thank Dr. Luca Albarello for useful discussion and suggestions, Roberto Cairella and Patricia Ney for their invaluable technical assistance.

Authors' contributions MF and SP were involved in patient care and provided clinical information and specimen. LP and IF performed FISH. RM performed laser capture microdissection. MGC and GG performed sequencing. MSL, AP, and CD wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. WHO (2019) Classification of Tumours Editorial Board, Digestive System Tumours, 5th edn. IARC Press, Lyon
2. Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) (2010) WHO classification of tumours of the digestive system, 4th edn. IARC Press, Lyon
3. La Rosa S, Ucella S, Molinari F, Savio A, Mete O et al (2018) Mixed adenoma well-differentiated neuroendocrine tumor (MANET) of the digestive system: an indolent subtype of mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN). Am J Surg Pathol 42(11):1503–1512
4. Manuel-Vazquez A, Ramia JM, Latorre-Fraqua R, Valle-Rubio A, Arteaga-Peralta V, Ramiro-Pérez C et al (2018) Pancreatic neuroendocrine tumors and intraductal papillary mucinous neoplasm of the pancreas: a systematic review. Pancreas. 47(5):551–555
5. Woischke C, Schaaf CW, Yang HM, Vieth M, Veits L, Geddert H, Märkl B, Stömmer P, Schaeffer DF, Frölich M, Blum H, Vosberg S, Greif PA, Jung A, Kirchner T, Horst D (2017) In-depth mutational analyses of colorectal neuroendocrine carcinomas with adenoma or adenocarcinoma components. Mod Pathol 30(1):95–103
6. Lloyd RV, Osamura RY, Kloppel G, Rosai J (eds) (2017) WHO classification of tumours of endocrine organs, 4th edn. IARC Press, Lyon
7. Marrache F, Cazals-Hatem D, Kianmanesh R, Palazzo L, Couvelard A, O'Tole D et al (2005) Endocrine tumor and intraductal papillary mucinous neoplasm of the pancreas: a fortuitous association? Pancreas. 31(1):79–83
8. Kadota Y, Shinoda M, Tanabe M, Tsujikawa H, Ueno A, Masugi Y, Oshima G, Nishiyama R, Tanaka M, Mihara K, Abe Y, Yagi H, Kitago M, Itano O, Kawachi S, Aiura K, Tanimoto A, Sakamaoto M, Kitagawa Y (2013) Concomitant pancreatic endocrine neoplasm and intraductal papillary mucinous neoplasm: a case report and literature review. World J Surg Oncol 11:75
9. Moriyoshi K, Minamiguchi S, Miyagawa-Hayashino A, Fujimoto M, Kawaguchi M, Haga H (2013) Collision of extensive exocrine and neuroendocrine neoplasms in multiple endocrine neoplasia type 1 revealed by cytogenetic analysis of loss of heterozygosity: a case report. Pathol Int 63(9):469–475
10. Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P et al (2017) Whole-genome landscape of pancreatic neuroendocrine tumours [published correction appears in nature. 2017 Sep 27]. Nature. 543(7643):65–71
11. Amato E, Dal Molin M, Mafficini A, Yu J, Malleo G, Rusev B et al (2014) Targeted next-generation sequencing of cancer genes dissects the molecular profiles of intraductal papillary neoplasms of the pancreas. J Pathol 233(3):217–227
12. Roy S, LaFramboise WA, Liu TC, Cao D, Luvison A, Miller C et al (2018) Loss of chromatin-remodeling proteins and/or CDKN2A associates with metastasis of pancreatic neuroendocrine tumors and reduced patient survival times. Gastroenterology 154(8):2060–2063.e8
13. Chung DC, Brown SB, Graeme-Cook F, Seto M, Warshaw AL, Jensen RT, Arnold A (2000) Overexpression of cyclin D1 occurs frequently in human pancreatic endocrine tumors. J Clin Endocrinol Metab 85(11):4373–4378
14. Biankin AV, Binakin SA, Kench JG, Morey AL, Lee CS, Head DR et al (2002) Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut. 50(6):861–868
15. Fritz S, Fernandez-del Castillo C, Mino-Kenudson M, Crippa S, Deshpande V, Lauwers GY, Warshaw AL, Thayer SP, Iafrate AJ (2009) Global genomic analysis of intraductal papillary mucinous neoplasms of the pancreas reveals significant molecular differences compared to ductal adenocarcinoma. Ann Surg 249(3):440–447