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Viewpoint

Mismatch repair proteins and microsatellite instability in solid pseudopapillary neoplasm of the pancreas

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Solid pseudopapillary neoplasms (SPNs) are rare solid pancreatic tumors mainly affecting young women. Despite the high percentage of favorable prognosis, they are considered as low grade malignant neoplasms, and metastases occur in 5%–15% of patients. Almost all SPNs (95%) have somatic activating mutations in the β-catenin gene [1]. β-catenin plays a crucial role in cell proliferation and differentiation via Wnt signaling pathway and interacts with E-cadherin and α-catenin for the regulation of cell adhesion and growth [2]. Another relevant genetic alteration in pancreatic neoplasms is represented by the DNA mismatch repair deficiency (dMMR). dMMR is strongly associated with a microsatellite instability (MSI) status as a result of an alteration in the lengths of microsatellites due to deletion/insertion of repeating units in tumor DNA [3]. dMMR was observed in approximately 1%–2% of patients with pancreatic adenocarcinoma [4]. To date, the "gold standard" for the evaluation of protein integrity of MMR is immunohistochemistry (IHC), showing an analytical sensitivity >90%, specificity of 100% and predictive value of 97% for microsatellite stability (MSS) and 100% for MSI [5]. The study aimed to investigate the expression of MMR proteins and the MSI status in pancreatic SPNs.

All pancreatic tumors collected between January 1997 and August 2017 at Unit of Pathology of Foundation IRCCS 'Casa Sollievo della Sofferenza' were reviewed by two expert pathologists and four cases of SPN were selected. The median patient age was 40 years (range 23–46) with 3 females and 1 male. The median tumor size was 7 cm (range 4–15). Three tumors were located in the tail and one in the head of the pancreas. Morphological diagnosis was supported by IHC analysis. Representative formalin-fixed and paraffin-embedded (FFPE) tumor blocks and, in three cases, non-neoplastic tissue blocks were available. MMR proteins analysis (MLH1, PMS2, MSH2 and MSH6) was performed by IHC with the automated platform Autostainer Link 48 (DAKO, Carpinteria, CA, USA) according to the manufacturer's instructions. Briefly, 3-µm thick FFPE tissue sections were deparaffinized in xylene, rehydrated in graded alcohols, washed in double-distilled water and treated with DAKO solution (EnVision FLEX Target Retrieval Solution, High pH 50 ×) for antigen retrieval. The slides were treated with primary monoclonal antibodies against MLH1 (clone ES05, diluted 1:50, DAKO), PMS2 (clone EP51, diluted 1:40, DAKO), MSH2 (clone FE11, diluted 1:50, DAKO), and MSH6 (clone EP49, diluted 1:50, DAKO) at 97 °C for 30 min. Antigen-antibody reaction was visualized using EnVision FLEX kit with diaminobenzidine as chromogen, slides were counterstained with hematoxylin and covered. In case of diffuse and homogeneous expression of all the four proteins, a proficient profile MMR (pMMR) was observed. Conversely, the loss of expression of one or more of these proteins indicated a dMMR profile.

MSI assays were performed on microdissected DNA from FFPE sections using TapeStation 4200 platform (Agilent Technologies, Waldbronn, Germany). A neoplastic area with more than 5% of neoplastic cells and non-tumor tissue were selected for each patient. Briefly, tumor and non-tumor cells were microdissected by using a dedicated blade, then DNA was extracted using QIAamp Mini Kit (Qiagen, Hilden, Germany) and was eluted in 30 µL of nuclease free water. DNA quantification was performed by dsDNA High Sensitivity Assay Kit on Qubit Fluorometer 2.0 (Thermofischer scientific, Carlsbad, CA), according to the manufacturer's instructions. An optimum of 20 ng was required to quantitative PCR amplification. PCR reaction was carried out to analyze Bethesda Panel (BAT-25, BAT-26, D2S123, D5S346, and D17S250) in five different reaction mix. PCR products were analyzed on Tape Station 4200 platform in association with D1000 reagents. Tape Station 4200 software analysis allowed to analyze results by comparing tumor electropherogram to the non-tumor one. One discordant locus defined low microsatellite instability status (L-MSI), whereas two or more discordant loci established high MSI (H-MSI). All samples were handled in compliance with the *Declaration of Helsinki*.

All tumors showed nuclear expression of β-catenin and TFE3, and focal and weak immunoreactivity for CD56 and Synaptophysin. Chromogranin resulted negative in all cases. All SPNs showed pMMR profile. The molecular assessment of the MSI status was concordant in 3 out of 4 cases. Conversely, in one case an H-MSI status was observed (Fig. 1).

Pancreatic SPNs are uncommon tumors of unclear cellular origin, accounting between 2% and 3% of all pancreatic neoplasms and 0.9% to 2.7% of exocrine pancreatic neoplasms, affecting predominantly younger women. In 2010, the World Health Organization

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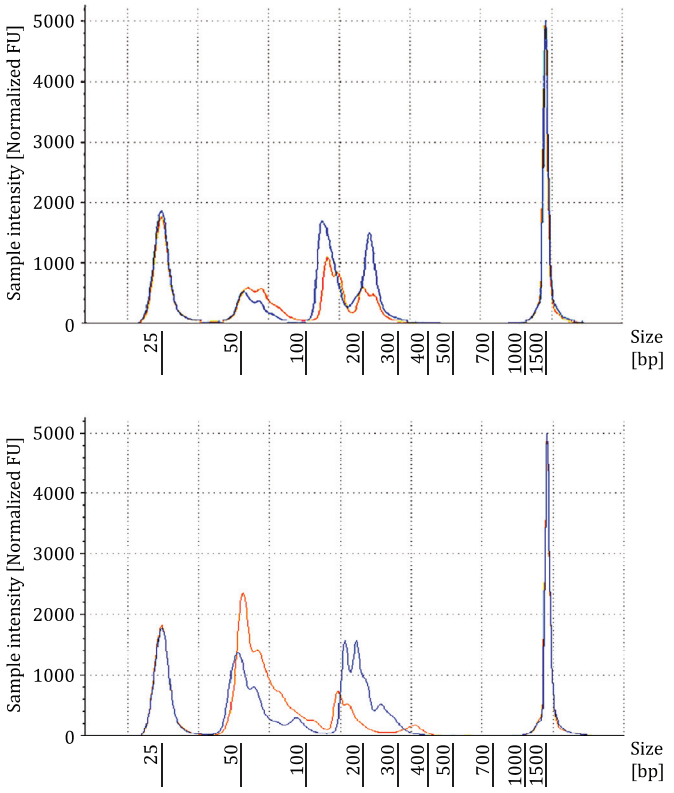

Fig. 1. Comparative analysis between normal tissue (yellow line) and tumor tissue (blue line) for locus D5 (upper) and D17 (below), showing instable electropherogram profile.

(WHO) classified SPNs as potential malignant neoplasms [6]. In fact, up to 15% of SPNs have an aggressive behavior, showing either invasion of adjacent tissues or distant metastases [7]. Because of their malignant potential, all SPNs should undergo surgical resection, with the evidence of a 5-year survival rate of up to 97% [8].

To the best of our knowledge, this is the first study focusing on the expression of MMR proteins together with the molecular MSI profile in pancreatic SPNs. Despite the limited number of cases, our results showed that the immunohistochemical expression of MMR proteins was retained in all SPNs. One case showed a discrepancy between IHC and MSI evaluation. However, this evidence is in accordance with literature, in which approximately 5% of cancers with pMMR on IHC showed a MSI status. In fact, an H-MSI status may depend on alterations in MSH3, PMS1, or EPCAM. These latter could be identified by molecular approach but not by IHC [9]. In addition, a major challenge in the use of the IHC approach in extra-colonic cancers is related to a problem of tissue fixation, which may influence the antigenicity, and to weaker internal control in respect to colon cancer specimens [4]. Notwithstanding the limited number of cases, our findings could be relevant to better understand the clinical behavior of SPNs and potentially useful for therapeutic purposes, in particular in the field of immunotherapy.

In conclusion, pancreatic SPNs are rare low grade malignant neoplasms whose clinical and pathologic features still need to be extensively studied. We found an H-MSI profile in one out of four SPNs. This finding paves the way for a new possible pathway that could be related to pathogenesis, clinical behavior and treatment choice of these neoplasms, and needs to be further evaluated on a larger number of cases.

Contributors

CC and Parente P conceived of the study. PF and MU performed the experiments. FF contributed for the technical assistance. CC and Pisapia P collected the pathological data. All authors contributed to the design and interpretation of the study and to further drafts and approved the final version of the manuscript. CC is the guarantor.

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Ethical approval

All information regarding the human material was managed using anonymus numerical codes. All samples were handled in compliance with the *Declaration of Helsinki*.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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