

February 21, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8011-Review.doc).

**Title:** Molecular pathology of intraductal papillary mucinous neoplasms of the pancreas

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Andrea Baldoni, Massimo Falconi

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript No:** 8011

The manuscript has been modified according to the suggestions of reviewers. Moreover we would like to specify that:

- 1) Format has been adapted to the style of the *World Journal of Gastroenterology*;
- 2) Revision has been made according to the suggestions of the reviewers as listed below;
- 3) New sentences are underlined in the text;
- 4) Modified or deleted sentences are ~~striketrough~~ in the test;
- 5) New references are added and other references are removed, and thus the numbering has been changed.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Massimo Falconi

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**REVIEWER 00672164**

1) *It lists genetic and epigenetic alterations reported in IPMN in a random fashion (irrespective of their prevalence and significance in malignant transformation). It is worth reorganizing the contents to emphasize alterations that are significant in the differentiation of:*

*- 1. IPMN vs. other pancreatic cystic neoplasms;*

**Response:** with regards to this point, we have focused our paper on IPMNs and the comparison with the other pancreatic cystic neoplasms is not the aim of the present work. We agree with the reviewer that it is of interest, but another different paper focusing on all pancreatic cysts should be considered.

*- 2. high-risk vs. low-risk IPMN lesions. In addition, if the authors could come up with recommendations on a panel of molecular assays that differentiate those lesions, it would be very helpful.*

**Response:** we have emphasized this aspect with the addition of a paragraph named “DISCUSSION” at the end of the paper, before the “CONCLUSIONS” (pag. 19), in which we have summarized the most significant molecular markers that are useful in differentiating malignant IPMNs from benign IPMN lesions.

2) *The manuscript may need professional editing by a native English speaker*

**Response:** the manuscript has been edited by a native American speaker (Mrs McGrath, a research nurse at MGH, Boston, USA)

3) *page 6: moderate -> intermediate-grade*

**Response:** the word “moderate” has been replaced with “intermediate”.

4) *page 7: Colloid carcinoma is not a common lesion in the skin*

**Response:** “and the skin” has been deleted as suggested.

5) *page 7: The oncocytic type is by definition of high-grade dysplasia based on its architectural complexity, but it may not be so proliferative*

**Response:** the sentence as been modified as requested and it now reads as follow: “originally described as a separate entity(19), this IPMN subtype shows ~~highly~~ proliferative cells associated with atypical cytology. ~~In many cases it~~ is associated with high-grade dysplasia.”

6) *pages 25-26: MUC5AC expression is a hallmark of IPMN, and thus, it is seen all IPMN lesions irrespective of histologic grades and epithelial subtype*

**Response:** the sentence regarding MUC5AC has been changed deleting “strong association with malignant transformation. MUC5AC expression is 84.7% in adenoma/borderline IPMNs and 92.4% in carcinoma IPMNs. Although MUC5AC in cystic fluid is not useful to distinguish high-risk cysts from low-risk cysts, the analysis of serum levels of MUC5AC can discriminate between high-risk cysts, with elevated MUC5AC, and low-risk cysts.” and adding “presence in all IPMN lesions, irrespective of histological grades and epithelial subtypes”.

#### **REVIEWER 00039635**

1) *I'd cut some of the introduction in the sub-sections when you describe general characteristics of the genes you describe in your paper*

**Response:** we have cut some of the generalities concerning genes; please refer also to consideration after response 2 to Reviewer 00159662. reduction of generalities concerning genes.

2) *What about your personal experience: even if it's a review, on my personal opinion your team personal approach after histological diagnosis could be very interesting resulting in more strength of the paper*

**Response:** We have added the sentence “Chemotherapy for patients with resected invasive IPMN is recommended; adjuvant treatment is advisable also in case of positive resection margins or lymph node metastases. Moreover lifetime surveillance is required also for noninvasive IPMN after partial pancreatectomy since the risk of local recurrence in the pancreatic remnant is about 8-10% and it may be the expression of a new metachrous tumor.”, at pag. 5 in the section “PATHOLOGY OF IPMNs”, according to Reviewer’s suggestion.

**REVIEWER 01204006**

*I encourage the authors to use some of the information listed in Table 3 to construct a practical diagnostic algorithm that integrates imaging and histologic findings and help guide practicing pathologists fine-tune their classifications of real-life cases.*

**Response:** we have created a practical diagnostic algorithm for the management of patients with IPMN diagnosed with imaging; we propose a possible help guide on the base of molecular genetics data presented in the paper for the discrimination between IPMNs that need surgery and IPMNs with a benign behavior which can be maintained under surveillance.

We thank the Reviewer for the suggestion that allow us to upgrade our paper.

**REVIEWER 00159662**

*1) Section "PATHOLOGY AND CLASSIFICATION OF IPMNs" should be divided in two: (i) Classification, (ii) Pathology*

**Response:** section "PATHOLOGY AND CLASSIFICATION OF IPMNs" has been divided in two sections: "CLASSIFICATION OF IPMNS" and "PATHOLOGY OF IPMNs" as suggested.

*2) The aim should be removed from the section "molecular genetics of IPMNs" and should be transferred to the section introduction*

**Response:** the aim has been moved from the section "MOLECULAR GENETICS OF IPMNs" (pag.6) in the section "INTRODUCTION" (pag.4).

In regards to the next points, we would like to point out that the paper is not addressed to highly capable molecular pathologists, but mainly to surgeons and gastroenterologists; then, we believe that a short description of each gene could be useful for a better understanding of the paper. We agree anyway with the Reviewer on the deletion of mutations in organs other than pancreas and on the reduction of generalities concerning genes.

*3) Sub-section "Kirsten ras oncogene": The generalities concerning kras should be removed since it is*

common knowledge (From “The most frequently... to ...codon 13 (21).)

**Response:** we have deleted the phrases “The most frequently mutated oncogene in IPMNs is” and “The cellular functions regulated by this protein are proliferation, motility, cytoskeletal remodeling and cellular survival. The activating point mutations of the gene in codon 12 abolish the regulated GTPase activity of the **Kras** protein.”.

4) Sub-section “PI3K/Akt” signaling pathway”: Only the phrases relative to IPMNs should remain. Notably: “In IPMNs, the frequency of the somatic mutation of the PIK3CA gene is 11%(27). In colorectal cancers, PIK3CA mutations generally arise just before or coincident with invasion(39), while, in IPMNs, seem to be a rather late event on the transition of these lesions to malignancy(27, 42).”

**Response:** only the phrases relative to IPMNs remained as suggested.

5) Sub-section “BRAF and the RAS/MAPK pathway”: Only the phrases relative to IPMNs should remain. Notably: “The rate of the somatic BRAF mutation described for IPMN is only 2.7%(21, 27). The BRAF mutation frequency is then low compared with those observed in malignant melanoma and colon cancers, but anyway the alteration of the Ras-Raf-MEK-ERK-MAP kinase pathway by BRAF mutation together with Ras mutation may play an important role in the tumorigenesis of IPMNs(21, 27); it is possible that tumors with both BRAF and KRAS mutations have an accelerated course in the development or progression(27).”

**Response:** only the phrases relative to IPMNs remained as suggested.

6) Sub-section “Telomerase Reverse Transcriptase Expression”: The generalities about telomerase should be removed. Notably from “Human telomeres are... to ... the pancreatic ones(54).”

**Response:** we have removed the sentence “Telomerase activation and up-regulated hTERT expression are involved in malignant cell transformation of many human tumors(49,54,55), including the pancreatic ones(56).”.

7) Sub-section “Hedgehog signaling pathway”: The generalities should be removed, From “The Hedgehog family... to ... and in its precursor lesions(62).”

**Response:** we have removed the phrase “its misregulation has been implicated in the development of a number of tumors, such as basal skin cell carcinomas(58), colorectal cancer(59,60), medulloblastoma(61), lung(62) and prostate(63) cancer and also in pancreatic cancer and in its

precursor lesions(64).”.

8) Sub-section “Cyclin-dependent kinase inhibitor 2A/p16”: The generalities should be removed from “CDKN2A is... to ... pancreatic carcinogenesis.”

**Response:** we have removed the phrases “including CDK4 and CDK6(68), in order to inhibit their catalytic activity and leading to reduced phosphorylation of the Retinoblastoma protein (pRb) and so G1 cell cycle arrest. Then, p16 indirectly” and “leads to entry in cell cycle and”.

9) Sub-section “TP53 gene”: The generalities should be removed from “The tumor suppressor... to ...hepatocellular cancer(78).”

**Response:** we have removed the sentences “Functional loss of p53 protein enables cellular survival and division in case of DNA damage, facilitating the accumulation of further genetic abnormalities(73,74). The accumulation of mutant forms of p53 in the nucleus can be considered a prognostic factor for gastrointestinal tumors as gastric cancer(77), colorectal cancer(78), pancreatic cancer(79) and hepatocellular cancer(80).”.

10) Sub-section “Deleted in Pancreatic Cancer locus 4 (DPC4)”. The generalities should be removed from “DPC4 tumor-suppressor gene... to ...of the cases)(84, 86, 87).”

**Response:** we have removed the phrase “can occur in a small percentage of carcinomas, such as breast, ovary, colon and biliary tract cancers(86,88), but it”.

11) Sub-section “Serine/threonine kinase 11 gene (STK11)”. The generalities from “STK11 gene... to ....various neoplasms(93, 94).” should be removed.

**Response:** we have removed the sentence “PJS is an autosomal-dominantly inherited disease characterized by hamartomatous polyps of the gastrointestinal tract, pigmented macules of the lips and buccal mucosa and predisposition to various neoplasms(95,96).” and the sentence “LOH at STK11/LBK1 locus has been also reported in sporadic cancers originating from breast, colon and ovary(100-102).”.

12) Sub-section “Brahma-related gene 1”. Introductory phrases should be removed from “BRG1, encoded... to ...pancreatic cancer(101).”

**Response:** we have removed the sentence “different types of cancer and abnormalities in its expression were observed in several neoplastic cell lines, such as in lung cancer, breast, brain, colon, ovarian cancer, and, less frequently, in pancreatic cancer(103).”.

13) *Sub-section “The S100 protein family”. a. Introductory phrases from “The members of the... to ...survival and invasion(117).” Should be removed. b. The following phrases should be removed “From Protein S100A4... to pancreatic carcinomas(124).” c. The following phrases should be removed “from Protein S100A11... to ...prostate cancer(130).”*

**Response:** we have removed the sentence “S100P is known to be expressed in breast cancer(115), in prostate(116) and in lung cancer(117).”, the sentence “An overexpression of S100A4 was detected in 41% of breast cancer(122), in 55% of gastric cancers(123), in 94% of colorectal adenocarcinomas(124), in 25% of esophageal squamous cell carcinomas(125) and in 95% of pancreatic carcinomas(126).” and the sentence “Expression of S100A11 can be detected in various tissues, as placenta, heart, lung, kidney, muscle and poorly in brain(129). S100A11 is found to be overexpressed in gastric carcinoma(130), in colorectal cancer(131) and in prostate cancer(132).”.

14) *Sub-section “Aberrant expression of microRNAs” a. The generalities concerning microRNAs should be removed from “microRNAs(miRNAs) are... to ...leukemia(143).” b. The part of the text “From chromosome 17q23.2... to...pro-apoptotic(152).” Should be removed c. The phrases from “miRNA-217 is a tumor... to ...and HOXB3(159, 160).” Should be removed as irrelevant*

**Response:** we have removed the sentence “Aberrant expression of miRNAs is frequently seen in human cancers, with both over and under expressed miRNAs in neoplastic cells(139), as cancer of lung(140), breast(141), prostate(142) and in the cancers of upper gastrointestinal tract(143)and colon(144) and in leukemia(145).”, the sentence “the resulting mature transcript has been one of the first oncogenic miRNAs (onco-miR) identified in human cancers(148).” and the phrase “have been described as upregulated in various solid tumors, including pancreatic cancer(134, 148, 153).”.

We removed the phrases from “miRNA-217 is a tumor... to ...and HOXB3(159, 160).” as suggested.

15) *Sub-section “Human Mucin genes expression” a. The generalities from: “MUCs are a group... to ...in*

*ductal adenocarcinoma." Should be removed*

**Response:** We have removed the phrases "having oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages", "Moreover, mucins are expressed in various tumor types(173-175).", "Alterations in the glycosylation of mucins, as aberrant glycosylation, leading at truncated oligosaccharide side chains such as the sialyl-Tn antigen, and incomplete glycosylation, resulting in the accumulation of core oligosaccharide structures such as Tn antigen(176), have been described in cancer(177)." and "**MUC1** is a transmembrane signal transducer glycoprotein, consisting of three distinct domains: an amino-terminal region containing a hydrophobic signal sequence and degenerated tandem repeats, around 30 to 90 almost conserved tandem repeats of 20 amino acids and a third domain with a carboxyl-terminal region containing degenerate repeats, a membrane spanning region of 31 amino acids and a cytoplasmic tail of 69 amino acids(178).".