Author's response to reviews

Title: Computational Prediction and Experimental Validation Associating FABP-1 and Pancreatic Adenocarcinoma with Diabetes

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Version: 2 Date: 2 December 2010

Author's response to reviews: see over
Dear Dr Harris:

We do appreciate the thoughtful feedback on our manuscript #6063149704269204 – “Computational Prediction and Experimental Validation Associating FABP-1 and Pancreatic Adenocarcinoma with Diabetes”. Please find below an itemized response to each reviewer’s comments.

Response to Reviewer 1 (David Morse)
1. Comment: Major Essential Revisions: “Although FABP-1 is expressed in renal proximal tubule cells which shed into the urine, it is doubtful that cells from PaC will also shed into the urine by this mechanism. However, circulating tumor cells (CTCs) in the blood have been found to express FABP-1 after enriching the sample for CTCs extracting and detecting FABP-1 mRNA, and has thus been proposed as a marker for CTCs (study not-referenced in the manuscript). Unfortunately, CTCs are only associated with metastatic disease and thus could only be used for diagnosis of advanced stages of cancer. Otherwise, being that FABP-1 is cytoplasmic and not found on the cell-surface, it is difficult to see the utility of FABP-1 as a diagnostic marker because it would be difficult to develop a targeted imaging probe against it. The authors should more clearly state/justify the proposed method that will be used for detection of this diagnostic marker in patients.”

Response: The reviewer correctly points out that we did not reference FABP-1 expression as a marker of colorectal cancer circulating tumor cells, and we have now done so. It should be noted, however, that serum FABP-1 has been detected in various human/animal studies, and that commercially available ELISA kits are available for serum FABP-1 detection. It can be postulated that FABP-1 enters the serum as a result of cell lysis or via secretion. A mention of these points has been added to the discussion section.

2. Comment: Minor Essential Revisions: “Figure 2 would be better presented in the results section”.

2. Response: Figure 2 was moved to the results section.
3. **Comment: Minor Essential Revisions:** “Page 12, 3rd paragraph - There is a typo, FABP1-1 should be FABP-1.”

3. **Response:** The typo was corrected.

4. **Comment: Discretionary revisions:** “Based on the representative images provided for Figure 2, a score of 1 represents weak staining (expression). Hence, only 20% of ‘PaC-DM’, 10% of all ‘PaC’ and 5% of ‘PaC no DM’ have moderate to strong expression of FABP-1. Since it is not clear that weak expression could be detected relative to expression in normal tissues in the blood or urine, the reported 50% expression of FABP-1 in PaC-DM possibly overstates the potential for diagnostic utility (although 20% coverage is not bad). As reported, this marker is not an absolute marker of diabetes associated pancreatic cancer, e.g. 5% of PaC with no DM moderately or strongly express this marker. Based on these numbers, 4 out of 5 (80%) of tumors expressing this marker will have associated DM (which is not bad). The authors may choose to use a more stringent, and thus more relevant, cutoff for IHC data when reporting the percentage of tumors covered by the FABP-1 marker.”

4. **Response:** We do appreciate Dr. Morse’s comments. In our limitations section, we now state: “When considered a binary variable, FABP-1 staining was defined as a score ≥ 1. A more stringent cutoff for FABP-1 staining would change our reported associated with pancreatic cancer. However, we do feel that in this pilot study, grouping pancreatic cancer groups by the presence or absence of FABP-1 staining was reasonable, given that only one normal sample stained positive for FABP-1 and that the degree of FABP-1
expression is likely to vary between tumors. The extent of FABP1 staining will be explored in future studies with more patients samples that are better phenotyped.”

Response to Reviewer 2: (Per Bendix Jeppesen)
1. Comment: “You may also ask if the numbers of sample (patents) is high enough to make the statement that the authors do in this paper.”
1. Response: We recognize that this is a pilot study and state accordingly in the paper. Further studies are needed to validate FABP-1’s association with pancreatic cancer.

2. Response: Corrected accordingly.

3. Comment: Minor Essential revisions: “Is there a reason why the authors don’t use Type 2 Diabetes instead of Diabetes mellitus?”
3. Response: The term “Diabetes mellitus” is used, as opposed to type II diabetes, in order to distinguish pancreatic cancer-associated diabetes from type II Diabetes. We have made changes throughout the text in order to clarify this distinction.

4. Response: Corrected, though we believe this error to be on page 17 rather than 15.

5. Comment: Minor Essential Revisions: “It would be nice if you could correct for the age difference between normal and cancer group, as we know that type 2 diabetes is
offend are related to age.”

5. **Response:** In the existing text, we addressed age as a covariate in the results and limitations section. Additionally in Table 3, there was no significant difference in age between the PaCnoDM and PaC-DM groups who stained for FABP-1. A statement to this effect was added to the results section.

6. **Comment:** Minor Essential Revisions: “Further explanations needed for fig 2. Please illustrate on the pictures, the main differences.”

6. **Response:** Changes made.

**Response to Reviewer 3:** (Juergen Schrezenmeir)

1. **Comment:** “The methodology of search for candidates resulting in FABP1 should be described more precisely to enable understanding the process of data analysis and the results of this search should be given in detail to enable understanding the basis of candidate selection. What was the scoring of FABP1 (between the given range of +1.0 and -1.0) compared to other genes, what was the ranking compared to other genes?”

1. **Response:** We have added the relative scoring and ranking of FABP-1 compared to other genes to the text.

2. **Comment:** “To further elucidate, whether diabetes may have been more or less likely to be associated with FABP1 expression by tumor tissue, the duration of diabetes in the 13 diabetes cases (based on patient’s histories) would be interesting to know.”
2. Response: We agree. Unfortunately, duration of diabetes was unable to be obtained in our retrospective chart review, thus we were unable to include this in our analysis.

3. Comment: “Furthermore the grade of infiltration of the pancreas of the cases compared to the cases with diabetes would be of interest, since diabetes might be due to the grade of infiltration, which may be related to the grade or kind of differentiation (that may be related to FABP1 expression).”

3. Response: The pancreatic adenocarcinoma samples in the FFPE TMAs were derived from patients who were considered to have disease that was resectable. Given that by definition this is a population without extensive tumor infiltration and the fact that 90% of the pancreas must be destroyed before endocrine symptoms develop, it is unlikely that tumor infiltration caused diabetes. Published literature supports this statement and notes the presence of pancreatic cancer associated diabetes in patients with tumors <1cm in size.

4. Comment: “FABP1 is suggested to be involved in the pathogenesis of diabetes via facilitating fat uptake, cellular trafficking, and chylomicron/VLDL production. This seems unlikely to take place to a significant extent in pancreatic tumor tissue, which is supplied by the nutrients via the portal vein. Therefore a secondary mechanism should be suggested in case the findings may be confirmed in later studies.”

4. Response: Our data reveal an association of FABP-1 with pancreatic cancer that is strengthened by the presence of diabetes. It does not reveal causation; we have no evidence to prove that FABP-1 is responsible for the pathogenesis of diabetes, nor do we
make this claim. Were FABP-1 found to participate in the pathogenesis of diabetes, one could surmise a mechanism of action, lipid trafficking aside, suggested by literature already referenced in our paper involving the PPAR-gamma pathway.

5. Comment: “Pg. 6 Which microarrays were used? (human genome wide? Which supplier?)”

5. Response: As stated in the paper, we used formal fixed paraffin embedded tissue microarrays created by the Department of Pathology at Stanford University.

6. Comment: “The authors state that a measure ranging from +1.0 (highest value found in pathologic tissue) to -1.0 (lowest value in normal tissue) was used to rank the genes expressed in diseased samples. This score should be given in the results as well as the scores of the other 10 genes ranking close to (higher and/or lower than) FABP1.

6. Response: Please see Comment 1 above.

7. Comment: “It is confusing that this measure evidently was not used as stated, but a comparison with pancreatitis and type 1 diabetes samples was used for selection.

7. Response: Rank change was used as stated, but I fear we did not express ourselves clearly. We have reworded the methods section for clarification of this point, and the role of other disease states for comparison.

8. Comment: “The sentence “we crossed the list” should be rephrased more precisely. Down to which RR or OR was this “crossing” done?”
8. Response: We have reworded this sentence in the methods section. Neither RR or OR were involved.

9. **Comment**: “Pg 7 The first sentence “given the beta cell ...” is not clear.”
9. **Response**: We have clarified this sentence as well as the rationale for comparison with type II diabetes susceptibility genes. This addresses Comment 10 below as well.

10. **Comment**: “Were all genes associated with DM or only those which were associated with beta-cell function and insulin resistance in pancreatic carcinoma? Why are type 2 diabetes susceptibility genes separately mentioned?
10. **Response**: Explained further

11. **Comment**: “Pg 15 Last sentence: “to have””
11. **Response**: We were unable to identify the sentence in question.

12. **Comment**: “Pg 16 Better rephrase: “Only one pathologist scored immunohistochemistry. He, however, has several years of experience.”
12. **Response**: Corrected as suggested.

13 **Comment**: “Table 1: The diabetes prevalence of 21% is in contrast to published data (45-65%). This should be discussed by the authors.”
13. **Response**: This was acknowledged in the original text in the limitations section of the paper.
We appreciate your careful evaluation of our work and hope that this revision meets with your approval. We believe these revisions have improved the manuscript’s clarity and organization. Thank you again for your interest in our work, and for the very thorough and thoughtful reviews. We await your review and consideration of our revised manuscript. We believe we have addressed the concerns of the three reviewers.

Sincerely,

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