Author's response to reviews

Title: Epidermal growth factor mediates detachment from and invasion through collagen I and Matrigel in Capan-1 pancreatic cancer cells

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Author's response to reviews: see over
Editor, BMC Gastroenterology

RE: Manuscript # 1831005154809254. Epidermal growth factor mediates detachment from and invasion through collagen I and Matrigel in Capan-1 cells

December 6, 2004

Dear Sir or Madam:

We appreciate the timely and insightful comments provided by the reviewers of our manuscript. We have endeavored to address each of their concerns, which are addressed in a point-by-point fashion below, and have revised the manuscript to reflect these changes. The changes in the manuscript are underlined and in red font. We have also performed certain additional experiments as suggested by the reviewers, with the additional data now incorporated in the revised version of the manuscript. With these changes, we believe that the manuscript has been strengthened significantly, and we hope that it will now be deemed suitable for publication in BMC Gastroenterology.

Response to Reviewers:

Reviewer 1 (Dr. Batra).

1. The statement “The inhibition of adhesion to collagen I…” in Results has been moved to the next section, as suggested. We have also modified this statement in light of the criticisms of Reviewer 2 (Point 5—see below).

2. The concentrations of the inhibitors used are now stated in the Methods section. We have also provided more details regarding the duration of growth factor treatment, as well as the duration of inhibitor treatment.

3. We agree that the statement “the lack of EGF secretion by these cells suggests that the drive to invasiveness in these cells is provided by cell-cell interactions” is not adequately supported by the data. Rather, it is one possible explanation, and we agree that alternative explanations also exist. This statement has been changed to reflect this viewpoint.

4. The points regarding FAK are speculative, and this section in the Discussion is now toned down to reflect this. Rather, the changes seen with actin rearrangement are suggestive of FAK involvement, but we agree that no direct data implicating FAK activation has been provided. This is an area for future research.

5. We have substantially expanded the analysis of our results and placed them in the context of the results of Stefani et al (Reference 26). As stated in the Discussion section, the difference in methodology with respect to growth factor treatment
duration on the adhesion assay is the most logical explanation for the differences in the results reported in our study compared to theirs.

Reviewer 2 (Dr. Kleeff).

1. The reviewer suggested using an additional pancreatic cancer cell line for some of our studies. We have now done so, and have chosen as a comparison the MIA PaCa-2 cell line. This cell line was isolated from a primary pancreatic tumor, in contrast to Capan-1 cells which were isolated from a liver metastasis of a pancreatic adenocarcinoma, thereby allowing us an opportunity to determine whether the de-adhesion and invasion effects of EGF can be related to the metastatic origin of the cells. We have now included data on invasion and adhesion assays with the MIA PaCa-2 cells, and have incorporated this data into the Results section, and revised the Discussion accordingly. The results of these studies are quite intriguing, and open up areas for future research.

2. The reviewer suggests that expression levels of all four EGF receptors be measured in Capan-1 cells using quantitative immunoblotting. Recently, Fauquette et al (Biochem J, October 5 2004, Epub) have shown that these cells express erbB2, whereas our data clearly shows EGF-R expression. We agree that demonstrating erbB3 and erbB4 expression by immunoblotting is missing from the literature. Nevertheless, this proposition would entail a significant amount of additional work, since expression levels are likely to change with the duration and dose of growth factor treatment. Time course and dose-dependency studies using antibodies against the four receptors would be necessary to provide a complete picture of erbB receptor family expression. In any case, expression levels and functional activation of these receptors are not synonymous, and showing one does not necessarily prove the other. This is an area for future research, particularly with respect to differential expression of these receptors in Capan-1 vs. MIA PaCa-2 cells.

3. The rationale for using heregulin alpha is now more clearly stated. In brief, we wished to determine whether a growth factor that is well established in other cell types to specifically target erbB2/erbB3/erbB4 shared the same phenotypic effects on adhesion/invasion as did EGF, which targets EGF-R and erbB2. The literature regarding the specificity of EGF for EGF-R and erbB2 on the one hand, and on the specificity of heregulins for erbB3/erbB4 on the other, is extensive (e.g. reviewed in Le et al, Apoptosis, 2002; 7:483-491; Bowers et al, Oncogene, 2001; 20:1388-1397).

4. It is well established that EGF is a ligand for EGF-R and erbB2 in numerous other cell types. Similarly, there is an extensive literature that heregulin-α binds to and activates ErbB3 and eB4 (reviewed in the references cited above). While it is true that none of these extensive studies were performed in Capan-1 cells (or any pancreatic cancer cells, for that matter), performing such validation studies would entail a significant investment in time and resources which would duplicate what
is well known in other cancer cell types. Such extensive studies would need to include demonstration of the activation/phosphorylation of these receptor tyrosine kinases, and the extensive downstream signaling cascades. These are all opportunities for future studies. We believe that the absence of such studies does not detract from the main points of our work.

5. Regarding EGF-R inhibitors or antibodies: During the course of this project, we were unable to find reliable research grade EGF-R inhibitors or blocking antibodies to perform the studies proposed. If and when such reagents become available, we plan to perform these studies. In the meantime, the absence of this data does not invalidate the main findings of our study, which strongly implicate the EGF-R in the EGF-mediated de-adhesion and invasion phenotypes demonstrated in the Capan-1 cells.

Again, we thank the reviewers for their timely and insightful comments. We accept the validity of their criticisms, as these all pertain to the fact that substantial additional work needs to be done with respect to the mechanisms involved in EGF and HRG-α-mediated effects on adhesion and invasion in pancreatic cancer cells. Our paper is among the first to bring into focus the links between EGF effects and adhesion/invasion capabilities in this cell type. Much additional work, including delineating the mechanisms of FAK-mediated cell movement, integrin signaling, and erbB receptor tyrosine kinase activation and downstream signaling in pancreatic cancer cells remain to be done. We hope that this study will be the first among a series to explore these mechanisms in detail, and the suggestions of the reviewers will certainly be helpful in the design of such studies. We believe that this work is an important advance in our understanding of the biology of pancreatic cancer cells that is particularly relevant today as there are now EGF-receptor antagonists entering clinical trials for the treatment of this malignancy.

Sincerely,

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