Author's response to reviews

Title: Transforming growth factor-beta suppresses metastasis in a subset of human colon carcinoma cells

Authors:

Neka Simms (nsimms@unmc.edu)
Ashwani Rajput (arajput@salud.unm.edu)
Elizabeth A Sharratt (lizrd716@hotmail.com)
Melanie Ongchin (melanie.ongchin@roswellpark.org)
Carol A Teggart (cteggart@unmc.edu)
Jing Wang (jjwang@unmc.edu)
Micahel G Brattain (mbrattain@unmc.edu)

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Author's response to reviews: see over
Dear Professor Roskelley,

Thank you for the continuing opportunity to have our work reviewed for publication in BMC Cancer. We appreciate the comments from you and the reviewers; and believe that they have led to an improved manuscript.

**To address reviewer #1’s concern regarding the flow of figure 2**, we rearranged the graphs and corresponding IHC images.

Page 10: KI-67 staining indicated no differences in the proliferation rates between FETα and FETα-DN implanted animals (FIG 2A and 2B). However, TUNEL staining was higher in tumors from FETα implanted animals thus, reflecting a larger number of cells undergoing apoptosis in FETα tumors as compared to FETα-DN tumors (FIG 2C). The apoptotic rate of FETα implants was 2.5-fold that of FETα-DN implants (FIG 2D).

Page 23: Figure 2. Enhanced cell survival capability of FETα-DN cells results in metastasis. Primary tumors established in animals orthotopically implanted with FETα and FETα-DN tumors were processed for (A-B) KI-67 staining and were analyzed to evaluate the proliferation rate. Primary tumors established in animals orthotopically implanted with FETα and FETα-DN tumors were processed for (C-D) TUNEL staining and were analyzed to evaluate the apoptotic rate. Both KI-67 and TUNEL images were captured at 200x magnification. Image J software was employed to quantify positive staining cells and the total number of cells. Statistical significance was determined using two tailed student’s t test with p value less than 0.05.

Figure 2 image file was altered to correspond to changes.

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**To address reviewer #2’s concern 1: the title was revised to include the term “subset”**.

Page 1: Title: Transforming growth factor-β suppresses metastasis in a subset of human colon carcinoma cells.

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**To address reviewer #2’s concern 2: The claim that modulating TGFβ has no effect on the invasiveness was removed by eliminating the following passages**.

Page 14: The selectivity of the effect on the colonization of distant organs as opposed to the invasion step of the metastatic cascade is consistent with the concept that different steps of the metastatic cascade develop different mechanisms for aberrant survival as reviewed in detail by Mehlen and Puisieux [33].

Page 15: Thus, it is not surprising that TGFβ signaling could result in the repression of metastasis without preventing the invasion step of the primary carcinoma.
Demonstration of more widespread validity with respect to cell lines in vitro: We have previously published work describing pharmacological induction of TGFβ receptors in vitro [9, 63] by a variety of HDAC inhibitors in several cell lines derived from breast, colon and pancreatic cancer in addition to the 2 cell lines used for the in vivo studies in this manuscript. Moreover, we recently published an additional paper showing that induction of TGFβ receptors resulted in the induction of cell death through in association with the modulation of survivin/XIAP [63] thus indicating that the observations made here are not rare. We have added this information to the discussion in addition to a discussion of the evidence for transcriptional repression of TGFβ receptors in clinical settings.

Page 16: We have utilized genetic modification of TGFβ receptors to show that TGFβ receptor mediated signaling is critical to the suppression of metastasis in the FET and CBS colon cancer models. The question arises as to the potential breadth of cancers in which TGFβ receptor modulation would be a factor and whether pharmacological modulation would be possible. Over the past 15 years we and others have shown that transcriptional repression of either RI or RII is seen in a variety of histological types of cancer including colon, breast, lung and pancreatic cells lines [7, 8, 9, 53, 54, 55, 56]. Along this line, several clinical studies have indicated that cancer progression is associated with loss of TGFβ receptors in types of cancers where TGFβ mutation is rare or in the case of colon cancer, in patient samples without microsatellite instability thereby implying a lack of mutation [57, 58, 59, 60, 61, 62]. More recently, we have shown that cancer cell lines with TGFβ receptor repression due to histone acetylation can be rescued by treatment with a clinical HDAC inhibitor candidate. Importantly, this pharmacological rescue results in TGFβ signaling dependent induction of apoptosis through disruption of survivin/XIAP mediated cell survival as seen both in vitro and in vivo in the 2 cell lines studied here as well as a pancreatic cancer cell line and 3 breast cancer cell lines [63]. Consequently, based on the broad range of cell lines showing TGFβ receptor repression, the clinical studies of cancer progression related to TGFβ receptor loss in cancers that rarely show TGFβ receptor mutations and the pharmacological responses of cell lines demonstrating TGFβ receptor transcriptional repression, the subset of cancers in which TGFβ receptor signaling potentially enables metastasis appears to be significant in a subset of cancers. Moreover, the mechanism of TGFβ receptor repression may be susceptible to pharmacological intervention [63].

To address the editorial request #1: the request for an animal ethics statement is included in methods section under orthotopic implantation.

Page 8: All animal work was done in accordance with the Institutional Animal Care and Use Committees (IACUC) regulations. Protocol number was 920M.

To address the editorial request #2: the author contribution list is included at the end of the manuscript according to journal guidelines.
Page 17: Authors Contribution

NS involved in experimental design, performed in vitro assays and IHC assays and drafted manuscript. AR performed in vivo orthotopic implantation experiments. ES performed histological slide preparation, histology assays and statistical analysis. MO performed in vivo orthotopic implantation experiments. CT performed tissue culture. JW participated in experimental design and data interpretation. MGB involved in experimental design, data interpretation, manuscript revision.

This manuscript has been revised and conforms to the journal style highlighted in the prior correspondence. Thank you for your time and efforts.

Regards,

Michael G. Brattain