Author's response to reviews

Title: Aberrations of TACC1 and TACC3 are associated with ovarian cancer

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Author's response to reviews:

We would like to thank the reviewer, Dr. Shelling for his review of this paper, and in particular, his appreciation of our "careful analysis" of the data. We have fully taken on board his constructive critiques and suggestions, and feel that these comments have certainly added in a positive way to our original article.

Please find below the itemized list of comments and minor essential revisions requested by the reviewer:

1. We have substituted "dHPLC" in place of "denaturing HPLC" and "WAVE HPLC"

Methods section:

2. We have properly referenced the SAGE analysis and referred to T-BO1 and IMH-343 as tissue and tumor microarray slides.

3. In response to the question of the potential that the normal siblings used for comparison in the genotyping: "With the relatively late age of onset of ovarian cancer, it remains possible that some of the siblings that we have labelled as normal may actually be carriers, and have not yet developed symptoms." In most cases, we selected individuals in the same generation, so that age related effects should be reduced. All changes detected in affected and "normal" were cross-referenced with the dSNP and EST databases.

Thus, in the results, we have qualified the "normal" status to "normal (non-symptomatic)", and in the discussion section we have made the following qualification: "With the relatively late age of onset of ovarian cancer, it remains possible that some of the siblings that have been classified as normal may actually be asymptomatic carriers. Thus, some of these novel polymorphisms may actually represent low penetrance modifiers of ovarian cancer risk, in a similar manner to BARD1 polymorphisms that were subsequently shown to be associated with increased breast cancer risk in the general population [37-39]."

Results:
3. We agree that even in short term cultures that were probably used in the reported SAGE analysis, HOSE cell line will exhibit changes in gene expression. However “normal ovary tissue” would not be an appropriate control per se, as the ovarian surface epithelium constitutes a relatively small percentage of the ovary (this has been a bone of contention with regard to so-called normal ovary tissue from commercial sources). To overcome this critique, we have added a picture of normal OSE immunostained with the TACC1 and TACC 3 antibodies in Fig 1 and referred to this in the results section:

"Representative images demonstrating positive or negative tumor staining compared to normal human ovarian surface epithelium are shown in Fig. 1"

4. With regard to the possible role of TACC3 as a new familial predisposition locus, we have added additional references to the link between 4p16 and the development of gynecological cancers, a link between uterine and ovarian cancer in published studies, including the Gilda Radner registry, the common embryonic origin of the tumor progenitors, and the association of BRCA1 and BARD1 mutations with the development of ovarian and uterine cancer in the same individual of related family members. However, in addition, we have used more cautionary language in our reference to TACC3 as a familial predisposition locus:

In the results, we have changed the last sentence to: "These data suggest that TACC3 may be a new familial predisposition or modifier locus for gynecological cancer."

In the discussion: "In the Gilda Radner registry, the risk for uterine cancer is approximately five fold higher than the general population, and this risk increases with the number of first degree relatives diagnosed with ovarian cancer [40]. A similar finding was observed in a separate genealogy-based study [41]. Indeed, a link between ovarian and uterine cancer may not be surprising given the common embryonic origin of the epithelium of the female reproductive tract i.e. the coelomic epithelium [42]. In an independent study, in one BRCA1 linked family, the daughter of a patient diagnosed with ovarian cancer not only had bilateral breast cancer, but also uterine leiomyomata [43,44]. In addition, a germ line missense mutation in the BRCA1 associated protein BARD1 can give rise to independent tumors in the breast, ovary (clear cell carcinoma) and endometrium (also a clear cell carcinoma) [37]. This connection is particularly intriguing considering the recent observation that the C. elegans TAC protein interacts directly with the C. elegans homologue of BARD1 [45]."

In the Abstract conclusion, we have also changed the wording from: "TACC3 could be a hitherto unknown genetic factor in ovarian tumorigenesis” to “TACC3 could be a hitherto unknown endogenous factor that contributes to ovarian tumorigenesis.

In the final conclusion, we have suggested that “TACC3 gene may be associated with a subset of familial ovarian/gynecological malignancies”

5. In regard to the use of Fisher's exact test for statistical analysis of the data: We have confirmed with the Chair of the Roswell Park Cancer Institute's Department of Biostatistics, Dr. Kepner, that this is the correct method to analyze the categorical data.
6. We have recognized the reviewers point regarding "random allele loss", by adding "During growth and progression, tumors can undergo a substantial amount of genomic rearrangement, including translocations, deletions and amplifications. Although many of these changes are random, due to the inherent genomic instability that can occur during tumor cell division, consistent abnormalities in the same or related tumor types can suggest that one or more genes in a particular region may be involved in the pathogenesis of the disease."

7. We have expanded on chTOG, and referenced two reviews on chTOG, and its interaction with TACC3:

"TACC3 has an evolutionarily conserved interaction with the microtubule associated proteins and mitotic regulators, chTOG [50], and Aurora A kinase, and can be phosphorylated by the latter (Reviewed in [51])"

8. Table 2:

We agree with the reviewer that the subdivision of tumors into subtype and grade noted in this tumor can result in small numbers that can result in skewed statistics. We had mentioned this in the results section:

"However, as it is more difficult to obtain large numbers of these less common types of ovarian cancer, it should be stressed that further analysis will be required to investigate whether relationships between tumor type will hold in a larger sample set."

In addition, based upon the reviewer's request, we have also added the following to the discussion section:

"Subdivision of the tumors suggested a difference in the distribution pattern of expression of the two TACC proteins, with TACC3 loss being more common in serous papillary carcinoma compared with clear cell carcinomas, while TACC1 staining was less frequent in endometroid than in serous papillary tumor cores. However, due to the relatively small numbers of the tumors in each category, particularly the rarer endometroid and mucinous subtypes, firm conclusions about the exact distribution pattern will require analysis of a much larger sample set."

9. Table 3:

We have adjusted this table and references in the text to the standardized nomenclature, as per the reviewer's request.

We hope that these additions and revisions meet with your approval, and that the manuscript is now acceptable for publication in your journal.