Author's response to reviews

Title: SDHA Loss of Function Mutations in a Subset of Young Adult Wild-type Gastrointestinal Stromal Tumors

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Author's response to reviews: see over
To: Dr. Britta Weigelt  
Editorial Office, BMC Cancer  

August 8th, 2012  

Dear Dr Weigelt,  

We thank you and the reviewers again for their helpful comments regarding our revised manuscript ‘SDHA Loss of Function Mutations in a Subset of Young Adult Wild-type Gastrointestinal Stromal Tumors’ that we would like to resubmit for publication in BMC Cancer.  

Please find below how we have addressed the reviewer 1’s comments. The new changes from the last version are now highlighted in yellow. The reviewer 2 was pleased with our answers for all his comments, so therefore nothing else to address there.  

1. In the first paragraph of the introduction the authors state "Loss of SDHB expression was first identified in GIST occurring in the context of CSS with germline mutations of SDHB or SDHC" and quote the article by Janeway et al from 2011. This statement is incorrect. Loss of SDHB expression in GIST was first reported by our group (Gill et al in 2010) who reported loss of SDHB expression in the GISTs of the Carney Triad and the subset of pediatric and adult GISTs which they resemble clinically and morphologically and proposed that negative staining for SDHB identifies a new and unique type of GIST now called the SDH deficient GIST.  

Reply: We apologize for this error. We have included the correct reference and have replaced the following sentence by “Loss of SDHB expression was first identified in GIST occurring in the context of Carney triad and in a subset of pediatric and adult GISTs with similar characteristics [9].”  

2. First paragraph on the methods - the authors started with 17 WT GISTs – were these selected to be SDH deficient GISTs? Our experience is that about 90 to 95% of pediatric WT GISTs are SDH deficient, but only about one quarter of adult WT GISTs are SDH deficient. The authors should explicitly state whether these GISTs were selected to be SDH deficient for inclusion in the study as I really would be surprised a consecutive series of 17 WT GISTs were all SDH deficient (unless perhaps they were all pediatric). If there was a selection bias towards pediatric GISTs this should be stated.  

Reply: Samples were selected only based on their WT status for KIT and PDGFRA. We have replaced the following sentence “Samples from 17 patients with KIT and PDGFRA WT GIST, all gastric in location, were included for analysis” by: “Samples from 17 patients with gastric GIST and selected on the basis of wild-type status for the KIT and PDGFRA genes were included for analysis.”  

3. In the third and fourth paragraphs of the discussion the authors seem suggest for GIST that SDHA mutation can be germline or somatic. This is very weak evidence for this assertion given that they have only sequenced three exons. As the authors would know for SDHA,SDHB,SDHC and SDHD mutated paraganglioma virtually all mutations are germline with a second somatic ‘double hit’ rather than being due to two somatic events and they simply do not have enough data to comment on whether or not this may be the same in GISTs.
**Reply:** We have deleted the following sentences: ‘However, it is interesting to note that an independent somatic mutation (Arg589Trp) was previously reported in one young adult patient with sporadic KIT and PDGFRA WT GIST, carrying a germline p.Arg31X mutation [16]. Therefore, we propose that at least for GIST mutation affecting the SDHA gene can be germline or somatic. Somatic mutation of metabolic enzymes are not unusual in cancer as demonstrated by the recent discovery in several tumor types of somatic mutations in IDH1 and IDH2 genes, encoding isocitrate dehydrogenases 1 and 2 respectively [37-39].’

4. In the final paragraph the authors recommend screening for SDHA mutation in SDH deficient GISTs. At face value this seems very logical conclusion. However it is not straightforward. We do not know if patients with SDHA-deficient GIST are at increased risk for the other tumors associated with SDHx germline mutations. We do know that patients with SDHA germline mutations with more than one tumor type have not yet been reported and that although the germline nature of such mutations are now well documented, the risk to mutation carriers is also unknown. Put simply there is insufficient data to know the clinical significance of an SDHA germline mutation. We think the penetrance is low but don’t know.

What do we do with an individual or kindred with SDHA mutation? Is there sufficient evidence to offer the same screening we offer individuals with SDHB mutation? These are very challenging issues and should in some way be acknowledged.

**Reply:** We have replaced the last sentences “Altogether, the results of this study confirm that at least the young adult patients with KIT and PDGFRA wild-type GISTs should be screened for germline or somatic mutations in the subunits of the succinate dehydrogenase complex II. Indeed, this may impact the follow-up of patients with germline mutation who have a potential increased risk of developing paragangliomas and additional GIST and also the future development of therapies targeting the hypoxia pathway in this specific subset of GIST.” by: “Genetic screening for SDHB, C and D germline mutations is recommended for patients with paraganglioma/pheochromocytoma and SDH deficient GISTs. At the time of this writing, it remains uncertain whether patients with SDHA-deficient GIST are also at increased risk for the tumors associated with SDHx germline mutations. The penetrance of SDHA mutations is also unknown. Therefore, further investigations are needed to clarify the clinical significance of a SDHA germline mutation and its impact in terms of genetic counseling.”

Figure 4f - as discussed in my last review, the SDHB staining in figure 4f looks like it has not worked as there is no internal positive control (and the tumour does not demonstrate the typical morphology of an SDH deficient GIST). The authors need to review the IHC from the GIST to confirm it was genuine negative staining with a positive internal control and replace this photograph. Is this really the same GIST in panel 4E as 4F. Also the caption for this figure does not clearly indicate what IHC is used in each panel.

**Reply:** We have retaken new images for 4F, higher power as well as to include the internal positive control (vessel wall). Yes, 4E and 4F belong to the same patient. We have now clearly indicated which immunohistochemical stain pertains to which panel. Thank you for pointing that out.

We hope that you will find our revised manuscript worthwhile for publication in BMC Cancer and are looking forward to hearing from you soon.
Sincerely yours,

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