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
Dear Dr Weigelt,

We thank you and the reviewers for their helpful comments regarding our manuscript “SDHA Loss of Function Mutations in a Subset of Young Adult Wild-type Gastrointestinal Stromal Tumors”. We have revised our manuscript accordingly and answered to each reviewer comments. All the changes from the initial version are highlighted in green.

**REVIEWER 1**

1. I have a major difficulty with how the concept of wild type GIST is ‘framed’. The authors are using the term ‘wild type GIST’ as if it were synonymous with SDH deficient GISTs. This is not the case – the type of GIST which is now known as succinate dehydrogenase deficient GIST is a distinct subtype of wild type GISTs. They have also failed to reference a large amount of the recent literature on SDHB and GISTs which should be rectified. Whilst I do not wish to be perceived as conflicted, much of this literature is from our research group. To explain the consensus in the literature at the moment, a subgroup of wild type GISTs form a unique clinicopathological entity which are defined by negative staining for SDHB but also exhibit quite different morphological and clinical features. It is this entity which we initially reported and referred to as the SDHB negative (or type 2 GIST) when we described the entity and introduced this concept in 2010 (ref Am J Surg Pathol 2010 636-644). The accepted terminology for this entity is now “SDH deficient GIST” (ref Am J Surg Pathol 2011 1712-1721). SDH deficient GIST accounts for between 5 and 7.5% of all gastric GISTs in unselected populations and the great majority of pediatric GISTs. All SDH deficient GISTs are wild type for KIT and PDGFRA, but not all KIT and PDGFRA wild type GISTs are SDH deficient. Therefore I really think that to be in keeping with current knowledge and terminology the authors have to use the terminology “Succinate dehydrogenase deficient GIST” throughout this paper instead of “pediatric and WT GIST” for the reasons we have previously stated Am J Surg Pathol. 2011 Aug;35(8):1245-7 and have been stated by others Am J Surg Pathol 2011,35(11):1712-1721.

**Reply:** We agree with the reviewer that the ‘SDH-deficient GIST’ term better reflects our study group and have thus rephrased the introduction and replaced the phrase ‘pediatric and WT GIST’ throughout the text. As suggested we have added the key reference ‘Am J Surg Pathol 2010; 636-644.’
2. The authors quote Pantaelo et al’s description of 2 GISTs with SDHA mutation, but not their follow up report of 2 further GISTS associated with SDHA mutation (ref: Am J Surg Pathol. 2011 Nov;35(11):1750-2). That is, Pantaelo et al’s group has in fact already reported 4 SDHA mutations associated with GISTs not 2.

Reply: We have mistakenly omitted the latest reference by Pantaleo, which is now included and the total of SDHA-mutated GISTs (n=4) is now rectified throughout the paper.

3. The authors do not really justify only sequencing exons 2, 9 and 13 in the 111 additional succinate dehydrogenase deficient GIST. There is insufficient information known about SDHA mutations to indicate whether there are mutational hotspots. Preferably all exons should be sequenced. I accept this is time consuming and if this is impossible to do, but if this can’t be done this should be listed as a major limitation of the paper which has implications have not been discussed. For example when the authors describe an exon 2 mutation in a 26 year old woman with bulky intraperitoneal disease, they cannot be sure that there was not a mutation in the germline in the other exons. This is a very important point because SDHB, SDHC and SDHD double hit inactivation associated with paragangliomas are almost always associated with germline mutation rather than being due to two somatic events. This is why SDHB immunohistochemistry is an effective screening strategy for SDHx mutation. It is very important to know whether SDHA mutation follows a similar pattern. However there is insufficient data to support the author’s implication that there is not another germline mutation (in keeping with Knudson’s two hit hypothesis).

Reply: We chose to focus our investigation to SDHA exons 2, 9 and 13 due to the results of massive parallel sequencing of the six cases from our series and of four other cases reported in the literature; which identified mutations in these exons. A full sequencing of 131 cases would be very time and money consuming and beyond the scope of this project. We agree with the reviewer, however, that this represents a limitation of the study and also acknowledge that we cannot exclude that 26 year-old man reported in our series may carry a germline mutation in the other exon. For this reason, we have added the following sentence in the discussion: “Moreover, since we have sequenced only SDHA exons 2, 9 and 13, we cannot exclude also the presence of a germline mutation in one of the other exons. “

4. Was immunohistochemistry, western blotting and sanger sequencing interpretation performed blinded to the other results? Not being blinded does not invalidate the results, but it should be stated if this is the case. This is particularly important because my blinded interpretation of the SDHA immunohistochemistry presented in figure 4c is that it is positive.
Similarly whilst the photographed area of SDHB staining in fig 4F is negative there is no internal positive control - perhaps the authors can illustrate an area with a positive internal control.

Reply: We agree entirely with the reviewer that this is an important point and we indeed have performed the review of the SDHA and SDHB IHC results blinded to the genotypic results. The selection of cases for Western Blot, however, was performed subsequently, cases being selected for validation based on the results of IHC and genotyping. We have now added a clarifying statement in the Material & Method to reflect this point.

5. Have these patients been described in other studies? If so this should be stated.

Reply: As suggested we have included now the information requested by the reviewer, which was omitted inadvertently from our initial draft of the manuscript. Seven of the pediatric patients and two of the young adults were included in our previous publication (Agaram NP Clinical Cancer Res 2008). This statement has now been added in the manuscript.

Reviewer 2

1. The authors refer to 2 previously reported cases of SDHA-mutant GIST (ref 13; Pantaleo et al.). In fact, these authors have reported SDHA mutations in 4 young adults with WT GIST (see Pantaleo MA, Nannini M, Astolfi A, et al. Am J Surg Pathol. 2011 Nov;35(11):1750-2 PMID: 21997697). This reference should be added, and 2 should be changed to 4 throughout the manuscript.

Reply: Thank you for pointing this out. Similar to our response above to the first reviewer, we have now added the most recent reference by Pantaleo and changed the total number of reported SDHA-mutated GIST in the manuscript.

2. In the background section, the authors mention that WT GIST shows consistent loss of SDHB expression. This is not true for NF1-associated WT GIST (see Wang JH, Lasota J, Miettinen M. J Cancer. 2011 Feb 16;2:90-3 PMID: 21479127). For clarification, I would suggest adding "exclusive of NF1-associated" to this statement and adding the above reference to the manuscript.

Reply: We have rephrased the introduction in order to explain more clearly the concept of ‘SDH deficient’ GIST, which replaces now through the entire text ‘pediatric and WT GIST’. See also similar reply to comment# 1 of Reviewer 1.
3. A limitation of this study is the fact that only 3 exons of SDHA were sequenced in 11 cases. Other than the small number of previously reported cases, there is no obvious reason why SDHA mutations could not occur in the other 12 exons. The authors have not formally excluded the possibility of SDHA mutations in these 11 cases, although the immunohistochemistry data (i.e., retention of SDHA expression) suggest they are indeed SDHA wild-type. The authors should mention this limitation in the discussion.

Reply: We chose to investigate exons 2, 9 and 13 because the massive parallel sequencing of six cases of our series and of four other cases reported in the literature identified mutations in the exons. In order to address this limitation, we have added the following sentence in the discussion section: “However, since we have sequenced only exons 2, 9, 13, we cannot exclude the unlikely possibility of a SDHA mutation even in cases showing SDHA protein expression.”

4. The authors mention “partial loss” of SDHA expression in the GIST with p.D38V (assessed with the Abcam antibody), whereas this tumor showed no SDHA expression by Western blot (using the Cell Signaling antibody). It would be interesting to know whether SDHA could be detected in this case by Western blot using the Abcam antibody. "Partial loss" of expression is difficult to assess using immunohistochemistry and is not generally a pattern that is observed in SDHx-mutant tumors. The authors should comment further on this finding.

Reply: We agree with the reviewer that the IHC may not be as reliable compared to western blotting, and that is the reason we have validated this result on western blotting. However, the SDHA antibody from Abcam (which we have tested previously) did not provide satisfactory results on Western Blotting, thus these findings were not included in the initial manuscript.

6. Based on the existing data regarding SDHx in paraganglioma, GIST, and renal cell carcinoma, it is highly unlikely that the SDHA mutation detected in the second case (p.D38V) is the only SDHA mutation in this tumor. Instead, there is probably a primary germline mutation, and the D38V mutation is likely the "second hit." The authors should provide this alternative hypothesis in the discussion, if not sequence the other 12 exons in order to attempt to identify the other mutation.

Reply: yes, as suggested we added a sentence in the Discussion to reflect the proposed ‘second hit’ mechanism as an alternative hypothesis.

We hope that you will find our revised manuscript worth for publication in BMC Cancer.

Sincerely,
Cristina Antonescu, MD
Director, Bone and Soft Tissue Pathology