Reviewer's report

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

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Reviewer: Anthony Gill

Reviewer's report:

This is an interesting article which contributes to our understanding of the relationship of mitochondrial complex 2 dysfunction (particularly SDHA mutation) and a specific type of GIST. However I have several major problems:

MAJOR COMPULSORY REVISIONS:

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 wildtype 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.

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.
Please rectify this 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).

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.

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

Have other SDH genes (SDHB,SDHC or SDHD) been sequenced on these patients as part of other studies? If so this should be stated.

Minor Essential Revisions:
It is not true to say that loss of SDHB expression results in inhibition of the degradation of HIF. Loss of SDHB expression is a marker that the mitochondrial complex 2 is not intact or functional. It is thought that dysfunction of the mitochondrial complex 2 leads to accumulation of HIF but inhibiting degradation of HIF.

I believe that any literature review on the area of SDH deficient GIST which does not quote the following articles is incomplete.


Doyle LAN, D; Heinrich M.C; Corless, C.L; Hornick, JL. Loss of SDHB expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumors: a comprehensive genotype-phenotype correlation study. Histopathology. 2012.

Discretionary Revisions:
The following articles which discuss SDHx mutations and renal neoplasia and paragangliomas should probably also be discussed as they are relevant to the use of IHC in screening for SDHx mutation but I feel less strongly about this. Because many of these articles are from our group and I do not wish to be considered conflicted or promoting our own articles, so these should be considered discretionary revisions.


**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have no competing financial interests.

Please note that our research group is actively performing research in similar areas.

Throughout my review I have declared this and I have assessed the paper in an unbiased fashion. When I recommend that information our research group has published on this area be considered, it is solely because it is relevant to this research report (not to promote our own research or views).