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: Jason L Hornick

Reviewer's report:

The authors report SDHA mutations in 2 young adult patients with KIT/PDGFRA wild-type (WT) GIST and demonstrate concomitant loss of both SDHA and SDHB protein expression, whereas SDHA expression was retained in WT GISTs without SDHA mutations. This study builds on recent prior reports. The main novelty in this manuscript is the demonstration of loss of SDHA expression, which has previously been documented in SDHA-mutant paragangliomas, but not in GIST.

Major Compulsory Revisions:

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.

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.

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.

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.
5. 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.

**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 declare that I have no competing interests.