Reviewer’s report

Title: Assessment of piRNA biogenesis and function in testicular germ cell tumors and their precursor germ cell neoplasia in situ

Version: 0 Date: 31 Jul 2017

Reviewer: Keyan Salari

Reviewer's report:

The authors of this study carry out a detailed analysis of the role of PIWI proteins and their associated piRNAs in the development of testicular germ cell tumors by characterizing their expression patterns among normal testis, testicular CIS, and TGCT tissue specimens. The authors reviewed that it had been previously reported that PIWI/piRNA expression is downregulated in TGCT compared to normal testis tissue. In this report, they extend the analysis of PIWI/piRNA expression to include testicular CIS specimens. Additionally, some functional studies were performed to explore the role of one specific PIWI protein family member, the PIWIL2/HILI isoform, in a TGCT cell line.

Major concerns:

1. The overall sample size of CIS and TGCT specimens is small. While the authors were able to take advantage of a the large dataset of normal testis tissue gene expression publicly available from the GTEx project, the number of CIS and TGCT specimens is limited. This becomes particularly problematic when one considers the heterogeneity within TGCTs with respect to histology and molecular genetics. Therefore there is concern that the findings in this report may not be generalizable to TGCTs broadly.

2. The functional studies were performed on only one cell line, TERA1, a cell line derived from a lung metastasis from a patient with embryonal carcinoma. It is difficult to draw any meaningful conclusions from the results of siRNA knockdown studies in one cell line, as these results may be entirely specific to this one cell line and not broadly representative of TGCTs.

3. The primary conclusion of the manuscript that downregulation of PIWI/piRNA genes in testicular CIS and TGCTs compared to normal germ cells suggests they are unlikely to be a driver of TGCT development does not appear to account for the possibility that PIWI/piRNA genes may play a tumor suppressor role in TGCTs. There are other examples of genes overexpressed in some cancer types and under expressed in others, where they can serve an oncogenic or tumor suppressor role, depending on the context. For example, it is conceivable that in the transition from normal germ cells to premalignant cells, loss of the PIWI/piRNA
pathway is necessary to allow for the activity of certain transposable elements that play a
tumorigenic role in TGCT.

Minor:

1. The term now adopted by GU pathologists and the 2016 WHO classification for the
precursor lesion of invasive germ cell tumors is "germ cell neoplasia in situ" (GCNIS), not
carcinoma in situ.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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