Reviewer's report

Title: WDR66 is a novel Marker for Risk Stratification and involved in Epithelial-Mesenchymal Transition of Esophageal Squamous Cell Carcinoma

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Reviewer: Rupert Langer

Reviewer's report:

The article of Wang et al presents the identification of WDR66 as a possible specific and prognostic marker for esophageal squamous cell carcinoma. The study delivers interesting results and is well performed, the study outline well-structured and logic.

Major Compulsory Revisions:

My major concern is that the tissue which was used for analysis is not characterized very well. The authors state that they obtained all tissue (fresh frozen) from the tumorbank of the CCC Charite. However, several times the authors mention the usage of FFPE tissue. Are these FFPE probes from the tumorbank as well? In contrast, the normal esophageal tissue was also fresh frozen? Can the authors assure that the investigated tissue (microdissected and non-microdissected) is really cancer (any histo-pathological expertise?).

Were the clinical follow up data associated with the probes also provided by the tumorbank? Is there any policy in the CCC for using these data (e.g. co-autorships, acknowledgements etc.)

The authors could provide a table with the clinicopathologic characterization of the ESCC collective which they used for survival analysis. Are they really all G1?

The second major issue is the statistics regarding the prognostic role of WDR66 expression. The authors should provide data about any association to established prognostic factors like T category, N category, grading. Moreover, had the factors included in the cox analysis prognostic impact on univariate analysis? It is somehow surprising that lymph node status had such a bad performance. Moreover, the authors include TNM stage, pT category and pN category in the same multivariate analysis. This approach is not quite correct, as TNM stage is composed of these two factors. How are the results, if the analysis is performed with TNM stage alone or alternatively pT and pN category.

Further comments:

Abstract: The abstract should be modified:
- Background: the aim is very vague (this is what biomarker research is in general..). The authors should be more specific.
- Methods: please specify: whole human gene expression profiling was
performed comparing normal with neoplastic tissue/ESCC. The second sentence could be shortened (e.g., whole human gene expression was performed after laser microdissection comparing..., “promising candidate”: what for;
- Conclusion: please explain shortly why WDR66 might be a novel drug target.

Introduction:
Although the article is well written in general, some lines and sentences would request a more careful editing. The paper would also become easier to read, if the authors would describe their stepwise approach in the introduction.

3rd paragraph: did the first microarray analysis really identify WDR66 as a biomarker for risk stratification or deliver information about its role for EMT? As far as I understood this step was used to identify the gene as being expressed in ESCC in contrast to normal tissue and other cancer types. Please clarify.

Materials and Methods (see above)
- What are “International Federation of Gastrointestinal criteria”?
- Next paragraph: “desired tumor cell or “BE” areas, better “NE”?

Results:
Could the authors provide information about the other differentially expressed genes?

Validation cohort: the authors have analyzed a series of different gastrointestinal cancers. Were they from all tumor stages/grading?

The authors describe the RNA In situ hybridization being applied on FFPE tissue. Did they fix the frozen tissue for that? This has not been described in the material and methods section.

Minor essential Revisions

Figure 1 has poor quality.

Discretionary Revisions/additional comment:

A last comment regarding authorship: reading the author’s contributions it is not clear why being involved in drafting/revising the manuscript (CM) should qualify as first authorship. Maybe the authors forgot to mention some more impact?

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I hereby declare that there are no competing interests regarding to the paper that I have been reviewing.