Peer Review File

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Reviewer A

Comment 1:

Authors have attempted to decipher the markers and mutations found in a rare subtype of breast carcinoma. However, there is lack of clarity in the article regarding the clinical usefulness of these markers and their implication on management of this subset of patients. It would be interesting to know whether detailed tests for various markers are needed to rule out the diagnosis of TCCRP in patients with a diagnosis of papillary lesions.

Reply 1:

Thanks for your suggestions. IDH2 and PIK3CA are both hotspot mutations of TCCRP. IDH2 is also the specific mutation site of TCCRP. In this paper, we have mentioned that Chiang et al. conducted gene detection on 13 patients by whole exon sequencing and targeted sequencing in 2016, and found that 77% (10/13) had IDH2 R172 mutation and IDH2 mutation was associated with the characterization of " reverse polarization ". Moreover, IDH2 hotspot mutation have not been reported in other types of breast cancer currently. Therefore, the diagnosis of TCCRP can be determined by results of sequencing and the combination with clinicopathological features, which is of great significance for the selection of clinical treatment modalities and prognosis assessment. We added the clinical usefulness of the markers and the implication as advised (see Page 9, line 177-179). At the same time, we also found other mutations in our research, but their indicative significance for TCCRP is not clear at present, and it is of great significance to supplement more cases with gene sequencing of TCCRP in the future.

Changes in the text:

Currently, IDH2 mutation is specific for the diagnosis of TCCRP, and the combination with clinicopathological features can clarify the diagnosis of TCCRP, which is conducive to the clinical treatment and prognosis evaluation.

Reviewer B

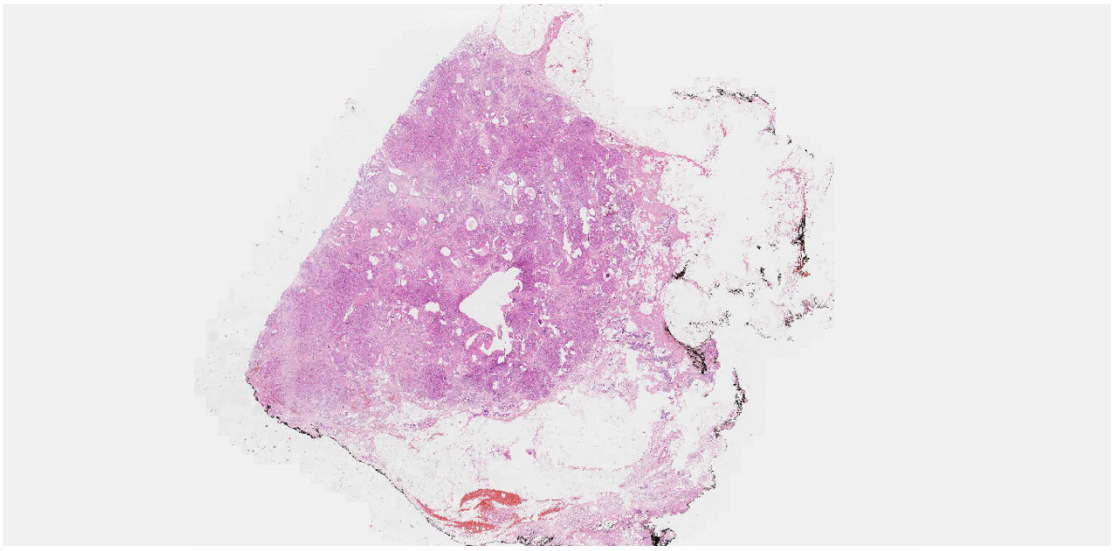
Comment 1:

Keeping in mind, this is supposed to be a very short case report, I would like the authors to add details about the dissection of these cases. Assuming the tumor samples were micro-dissected and DNA was extracted? Then the details of WES methodology, bioinformatics pipeline, and filtering criteria are completely missing. Have normal tissues been dissected and run with the tumor samples? If not, then the filtering criteria of variants must be stated. I am assuming there are more than 3 variants are found, in that case, a small Table as a Supp Table should be added for samples, variants, the

Allele frequency of those variants. And this list should not be only restricted to the common ones for these tumor types.

Reply 1:

Thanks for your advice. The tumor submitted for WES after surgical resection contained more than 90% of tumor tissue, with only a small portion of normal tissue (see the figure below), and normal breast tissue was also submitted for examination as control samples. Therefore, although there is no sample filtering and screening process, it is reasonable to believe that the genetic test results are accurate and reliable. According to your suggestion, we have attached the specific gene mutation results and mutation frequency of the gene test as Table S1.



Changes in the text:

Table S1. Gene mutations of Case1 and Case2 using whole exome sequencing.

Table S2 Clinical history, immunohistochemistry, gene mutation and follow-up information of the patients.

Comment 2:

Has Chromosomal aberrations been analyzed? If yes, then is it possible to provide a summary of those changes for these two samples as a Figure? If not at least should be mentioned in Results what are those changes.

Reply 2:

Chromosomal aberrations were not analyzed and were added in the results (see Page 6, line 100) and (see Page 7, line 120).

Changes in the text:

the results showed concurrent… (Table S1). Chromosomal aberrations were not analyzed. The patient was alive…

the results …hotspot mutations (Table S1). Chromosomal aberrations were not

analyzed. All of the results confirmed…

Comment 3:
Figures for IHC are fine except the scale bar is missing. This should be added.
Reply 3:
The scale bar of IHC was added to the figures (see Figure Legend: Figure 4).
Changes in the text:
The immunohistochemistry results of Case 1 and Case 2. (A, ×60, scale bar= 200μm)

Comment 4:
Details of IHC are missing. At least the antibody details (clones and company) should be stated in Method.
Reply 4:
Thanks for your advice. The antibody we used were reliable and standard. We were also aware of this question and were glad to add this content. But we have to notice that "method" is not requested according to the journal requirements of case report of Gland Surgery. Therefore, we decided not to add method part as required.
Changes in the text:
No text was changed according to this advice.

Comment 5:
Discussion is long, it can be cut it down, and concise the main findings. The first paragraph of the Discussion is kind of stated in the Introduction already. In Discussion, in Line 155, "the origin of the breast" could be expanded and stated clearly what are they referring to here.
Reply 5:
Thanks for your advice. Firstly, we cut down the repeated part of the first paragraph of the Discussion and stated the remaining content in the introduction (see Page 4, line 45-49). Then, "the origin of the breast" was expanded as advised (see Page 8, line 151-153).
Changes in the text:
It was first reported… (BTRPTC) "(1). Since subsequent studies found that it lacked the unique histologic and genetic properties of PTC, Masood et al. proposed that the terminology should be changed to " Tall cell variant of papillary breast carcinoma (TCVPBC) "(2). In 2016, Chiang et al. pointed out that the tumor showed solid and papillary structures lined by columnar epithelial cells with "reverse polarity" significantly, and suggested it be renamed " Solid papillary carcinoma with reverse polarization (SPCRP)" (3). In 2019….
It is suggested that the simultaneous staining of GATA-3 and SOX-10 can improve the

sensitivity of TCCRP to justify the origin of breast and distinguish from other carcinomas including metastatic thyroid carcinoma, etc.

Comment 6:

One way of cutting down words about the variants of IDH2 and PIK3CA mutation which other found, the authors potentially can add them in the Supp Table they already have. When they say positive for mutation, they can write the variant those other papers identified. Then while they are writing the findings from others, simply refer to that Table without stating every single study and their identified variants.

Reply 6:

Thanks for your advice. We have revised the manuscript as advised (see Page 6, line 98-100) and (see Page 7, line 118-120).

Changes in the text:

Molecular genetic analysis was conducted using WES, and the results showed concurrent IDH2 p.R172S and PIK3CA p.H1047R hotspot mutations (Table S1).

Molecular genetic analysis was conducted using WES, and the results showed concurrent IDH2 p.R172W and PIK3CA p.H1047R hotspot mutations (Table S1).

Comment 7:

One of the points the authors made about the diagnostic point of view is identifying IDH1 mutation through IHC. I found this clinically very important. Can they run IHC on their samples and prove their one point is that it is basically possible although they have only 2 cases? The other point that is not cleared from their discussion is that did all the study found IDH2 mutations? If I look at the Supp Table, it seems like most studies recorded IDH2 is a common mutation for TCCRP however some did not. If they want to make this claim as a diagnostic tool, they need to justify whether there is any reason why those few studies didn't find IDH2 mutation? Any technical limitations? Or it was simply absent. Could either PIK3CA or IDH2 possibly be a diagnostic tool instead? If any of those are present, then there are more clear guidance for pathologists that it is TCCRP. The issue with PIK3CA is that may be there might not be a good antibody available for IHC and PIK3CA is very common mutation in breast cancer overall including papilloma, esp H1047R. This point needs to be clarified in discussion to make a stronger argument.

Reply 7:

Thanks for your questions and suggestions. First of all, the IHC results we mentioned were studied by Alsadoun et al. and Pareja et al. of which IHC and WES were both conducted in 9 and 15 cases (more than 3 cases). In addition, the experimental design of these two research also included the grouping of samples from other papilloma patients such as EPC and IPC. By comparing the detection results of IHC and WES in

different groups, it was proposed that IDH2 could be used as a specific mutation site of TCCRP, and IHC detection of IDH2 mutations had good sensitivity (67% and 93%, respectively) and specificity (100%). Indeed, not all TCCRP cases have IDH2 mutations, and a higher proportion of PRUNE2 and ACACB mutations also have been found in IDH2 wild-type cases. But in our cases, PRUNE2, ACACB and other common mutations mentioned in the literature before were not found. We do not consider it the result of technical limitation. The mechanism of IDH2 mutation on the pathogenesis of TCCRP, whether other genes have a clear diagnostic significance for TCCRP, and whether IDH2 mutation is related to other gene mutations still need more follow-up cases and basic research to support. Finally, the content related to PIK3CA has been supplemented in the text as suggested (see Page 9, line 182-183).

Changes in the text:

Relatively, PIK3CA mutation does not appear specific in TCCRP which indeed can also be found in various types of breast cancer including papilloma.

Comment 8:

Minor points: Some typos with regards to “author name et al. “ are inconsistent. Some et al have dot some do not. Some space issues are also there when the reference number was added at the end of the sentence.

Reply 8:

Thanks for your advice. We have carefully checked these points again and revised the manuscript as advised (see Page 4, line 49).

Changes in the text:

"Solid papillary carcinoma with reverse polarization (SPCRP)"(3)

Comment 9:

Name of the genes ideally should be in italic across the manuscript unless the journal requirements says otherwise.

Reply 9:

Thanks for your advice. We have carefully checked these points again and revised the manuscript as advised.

Changes in the text:

IDH2, PIK3CA, R172S, R172G, R172T, PRUNE2, ATM, KIT, RET, BRAF.