

Peer Review File

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Reviewer A

It was a pleasure reviewing the manuscript ": BRAF, C-KIT, and NRAS mutations correlated with different clinicopathological features: an analysis of 691 melanoma patients from a single center" by Ren et al.

The manuscript is interesting but presentation is somewhat confusing. The reader has to go back and forth to understand what the authors are trying to convey

I have following suggestions:-

1. Avoid abbreviations without spelling them out. Eg. ALM in abstract

Reply: We have replaced “ALM” with “acral-lentiginous melanoma” in the Abstract (Page 2, Line 33).

2. Page 6 line 111 "And among 186 mucosal melanomas, 65 located in gastrointestinal tract, 53 in respiratory tract, 63 in urogenital tract, and 5 in conjunctiva." and Page 8 line 147 and 148-- "And gastrointestinal tract mucosal melanoma more likely had BRAF mutation than respiratory tract, urogenital tract and conjunctival melanoma ($P < 0.001$)." Are the authors stating this is the primary site of mucosal melanoma? This needs to be clarified

Reply: Yes, this is the primary site of mucosal melanoma. And we further clarify the statement in the part of Result (Page 7, Line 126-128; Page 9-10, Line 183-188).

3. While correlating clinical features please correlate cutaneous and mucosal differently in text as done in table. While reading the text the correlation text it doesn't become clear the authors are talking about cutaneous or mucosal or the whole group.

Reply: According to the Reviewer's suggestion, we have modified the part of clinicopathological features of different gene mutations in Results. We separately evaluate the clinicopathological features of BRAF, C-KIT and NRAS mutations in whole group, cutaneous and mucosal melanomas. The revised statement is located in Page 8-10, Line 162-188.

Reviewer B

The aim of the study is clear in reporting on the frequency of BRAF, C-KIT and NRAS mutation status of a large collection of melanoma patients ascertained within the one Institution. However, the clarity of the text could be much improved. The presentation of numbers to reflect frequencies in the Tables should be complemented by percentages. This would then make the text understandable.

Specific Comments:

1. The p-value results for the statistical analysis should be included in the abstract wherever there is “more likely”

Reply: According to the Reviewer’s suggestion, we have added the data of P-value in the abstract (Page 2-3, Line 33-45).

2. In the abstract,

“Statistical analysis showed that younger patients had a higher BRAF mutation rate than the older.”

You have to state here what younger and older are in the context of this paper i.e. under 60 years and over 60 years (The Table P = 0.000)

Reply: We have modified the statement in the abstract (Page 2, Line 33).

3. Page 4 line 53, “approved” should be approval

Reply: We have revised the word (Page 4, Line 59).

4. Page 6 line 108, “ ... ranged from 20 to 89 years ... ” this should be included in Table 1.

Reply: We have added the range and median of age in Table 1.

5. Page 6 line 109, “... ratio of male to female was 0.89.” this would be easier to understand as a % value and the data in all the Tables should also be presented as a % of the numbers to be placed in brackets next to the actual number as is commonly done in other papers.

i.e. 47.2% male/52.8% female OR just state 47.2% male

Moreover, in Table 1 for the cutaneous melanoma column it is 53.5% male/46.5% female and for the mucosal melanoma column it is 31.2% male/68.8% female. It may be of interest to highlight the changes in % of males when divided in this way.

Reply: According to the suggestions from the reviewer, we have revised the statement of ratio of male and female patients in the Results (Page 7, Line 122-123), and added the data of percentage value in Table 1. The relatively higher proportion of female patients in mucosal melanoma was related to the influence of patients with tumors in the female genital tract (Page 7, Line 128-130).

6. The data in paragraphs on Page 6 and 7 under the heading “Clinicopathological characteristics” should be tabulated and expressed as % even if it were included as an additional supplementary Table.

i.e. Among 361 cutaneous melanomas, 18 located in head and neck (5.0%), 56 in trunk (15.5%), 51 in limbs (14.1%), and 236 in extremity (65.4%) .

And among 186 mucosal melanomas, 65 located in gastrointestinal tract (34.9%), 53 in respiratory tract (28.5), 63 in urogenital tract (33.9%), and 5 in conjunctiva (2.7%). And 109 of the 547 primary patients (19.7%) involved lymph node metastasis at diagnosis.

In 361 primary cutaneous melanomas, acral-lentiginous melanoma (ALM, n=212; 58.7%) was the most common histological subtype, followed by superficial spreading melanoma (SSM, n=91; 25.2%), nodular melanoma (NM, n=50; 13.9%), lentigo maligna melanoma (LMM, n=5; 1.4%), and others (n=3; 0.8%). The median Breslow thickness of all lesions was 3.8 mm. Ulceration was found in 110 patients (30.5%). The detailed information of clinical and pathological characteristics in cutaneous and mucosal melanoma groups was shown in Table 1.

Reply: Thanks the advice of the reviewer. The data in paragraphs on Page 6 and 7 under the heading “Clinicopathological characteristics” were added the percentage value (Page 7, Line 124-135).

7. Page 7 line 122, “...(24.0%, Table 1).” This 24.0% is not in Table 1, see comment 5. This same point for C-KIT (9.4%, Table 1)

Reply: We have added the data of percentage value in Table 1.

8. Page 8 lines 140/141 “... the frequency of BRAF mutation in younger patients (< 60) was statistically higher than that in the older (≥ 60) (31.3% vs. 17.2%, P<0.001).” These % numbers are not listed in Table 2 and it is confusing the way they are presented.

For BRAF mutation:

there are $66 + 103 = 169$ under 60; the % here is 39.0% mutant and 61.0% Wt

there are $34 + 158 = 192$ 60 or older; the % here is 17.7% mutant and 82.3% Wt

The inconsistencies between the authors calculations on % should be considered but it will likely not change the P-value they present.

Reply: According to the suggestions of the reviewer, we have modified the part of “clinicopathological features of different gene mutations” in Results. The data in Page 8 lines 140/141 were evaluated in all 691 patients (31.3% vs. 17.2%), while the data in Table 2 were evaluated in 361 cutaneous melanomas (39.0% vs. 17.7%). To further clearly elucidate the landscape of mutations, we separately describe the features of BRAF, C-KIT and NRAS mutations in whole group, cutaneous and mucosal melanomas (Page 8-10, line 162-188), and add the data of percentage value in Table 2 and Table 3.

9. Table 2 should have (x%) included as much as possible next to the actual numbers to make the Table more understandable and to be consistent with the text. Significant P-values should be presented in Bold type.

Reply: We have added the data of percentage value in Table 2 and Table 3, and significant P-values have been presented in Bold type.

10. Page 8 line 153, “... was lower than that in the older (P=0.006)” The P-value for this cell in Table 2 is .001. Why the inconsistency?

Reply: The data in Page 8 line 153 were evaluated in all 691 patients, while the data in Table 2 were evaluated in 361 cutaneous melanomas. And we have separately described the features of BRAF, C-KIT and NRAS mutations in whole group, cutaneous and mucosal melanomas in Results (Page 8-10, line 162-188).

11. Page 9 line 159, “.. location in mucosal melanoma.” This data is in Table 3 and should be cited as such. At present Table 3 is not cited in the text? Similarly to the comment above, Table 3 should also have (x%) included next to all the numbers and significant P-values in Bold type.

Reply: Thanks the suggestions of the reviewer, we have cited Table 2 in the text (Page 10, line 188). And we have added the data of percentage value and significant P-values have been presented in Bold type in Table 3.