Reviewer's report

Title: Allelotyping identification of genomic alterations in rectal chromosomally unstable tumors without preoperative treatment

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Reviewer: kristina lagerstedt

Reviewer's report:

Romain and coworkers have investigated the frequency of allelic imbalance (AI) in relation to treated or untreated rectal tumors and compared the findings to AI in left colon tumors. The research question is valid and the comparison between rectal and distal colon tumors interesting. However, I do have some major and minor remarks.

Major Compulsory Revisions:

1) The panel of 33 markers used in this study does not generate sufficient information to draw conclusions about quantity of global genomic alterations in relation to progression. The authors also conclude that colon and rectum are separate entities based on limited genetic analyses. As stated by the authors themselves in the discussion, at least parts of the data should be validated by an extended analysis e.g. by array CGH of at least a number of tumor samples representing different clusters. (I recommend Tsafir D et al Cancer Res 2006).

2) Statistical analyses: In the paper Kaplan-Meier curves and log-rank tests for several microsatellites were performed, however no information is given regarding the correction for multiple testing. If no such correction was performed, it should be added. Furthermore, the survival analysis was done without adjusting for other confounding factors such as age and gender.

3) I did not find an aim of the study in the background, but parts of results and discussion. These parts should be moved/removed and replaced with an aim. Further, conclusions in the discussion and abstract are not convergent. I find it difficult to understand what the final message of this study is.

Minor Essential Revisions:

1) Were none of the 151 left tumors treated preoperatively? Please clarify! The data available from analyses of left colon tumors are very limited.

2) The authors should include the amount of tumor cells present in the samples (although previously reported).

3) Clustering: the authors need to specify algorithm and distance metrics.

4) How many tumors showed MSI (since only 1.9% was MSI-H)?

5) The authors should specify when different statistical tests were applied, e.g. when did they use the Mann-Whitney test?

6) Results, section Mean Global AI frequency, last 3 lines: “This difference
remained significant (39 %, P<0.05) after removing tumors that had lost all Als after preoperative treatment (n=4/24). Were Al checked prior to treatment? Please clarify.

7) Results section, first paragraph: the authors should include at least one reference when they state that the clinical data is in accordance with the literature?

8) Results section, section Potential survival markers for tumors without preoperative treatment: D5S340 should be D5S430?

9) Discussion, section Altered loci and associated genes: check marker loci in relation to referred genes e.g. D8S284 is not close to Dusp26 (D8S284 is located on 8q24 and Dusp26 on 8p12).

10) Discussion, Conclusion “the progression is not related to the quantity of genomic alterations”: this conclusion cannot be drawn from the data presented and the statement should be toned down.

11) Figure 4A: it is not possible to identify different markers and thus, one cannot evaluate distribution of data manually.

Discretionary Revisions:
1) Background, paragraph 5, allelotyping is described repeatedly and the text could be condensed
2) It would have been interesting if the authors presented more data on the differences and similarities between treated and untreated tumors
3) Table 1: the proportion of angioinvasion is reversed in preoperatively treated patients compared to the untreated and control cohort – can this affect the results?

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests