OPEN PEER REVIEW

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Peer Review Report for "Expression and Functional Assessment of Some Featured Coding and Non-coding RNAs Encoded by 8q24 Chromosomal Region in CML Patients"

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Submit Date: 14 Jan 2021
Revised Date: 28 Jan 2021
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Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0)

23 Jan 2021

Dear AE,
The manuscript entitled “Expression and functional assessment of some featured coding and noncoding RNAs encoded by 8q24 chromosal region in CML patients” has potential issues to be addressed. In my opinion, the manuscript is not acceptable for publication in the journal at this format and major corrections are needed.
1- The manuscript needs language editing extensively.
2- Introduction is too long (around 800 words). A common mistake is that authors are aiming to present a full review of the literature in their field. What is necessary instead is to reference those aspects that are needed to contextualize the problem that your study is solving. Introduction typically occupies 10-15% of the paper.
3- Symbols for genes are italicized. The formatting of symbols for RNA and complementary DNA (cDNA) usually follows the same conventions as those for gene symbols. Gene names that are written out in full are not italicized. Please follow this guideline through the manuscript.
4- Both grant ID and ethical approval number need to be included in the manuscript.

5- Patients' demographic and clinical presentation should be summarized as a table.
6- In silico analysis should be described in the methods section.
7- The data of in silico analysis should be discussed in the results and discussion. In this manuscript, the presentation of in silico data is not acceptable. It is just the output of datasets and webservers.
8- The BCR-ABL gene is not the type of mutation that is inherited from patient's parents. It is a type of somatic mutation, which means patients are not born with it and get it later in life. BCR-ABL is found in almost all patients with chronic myeloid leukemia (CML). The BCR-ABL gene is also found in some patients with a form of acute lymphoblastic leukemia (ALL) and rarely in patients with acute myelogenous leukemia (AML). As, in this project the researchers focused on CML type, how did they confirm included cases as CML type? Additionally, BCR-ABL test usually analyses the bone marrow aspiration and biopsy. However, in this study, blood samples were taken from enrolled patients. Could you please clarify if there is any concordance between the results obtained from these two procedures? If yes, why did you prefer the blood than the bone marrow? Please add all the information in the manuscript.
9- Author should present the expression data ($2^{-\Delta Ct}$) as Mean ±SD. Besides, the author should explain how much gene expression level of a target gene changes between Patients and Control groups (Fold change).
10- The degree of P-value should be also added through the result section.
11- The gene expression data related to MYC has been missed by the author in result section
12- In figure1, the error bars are big, mostly. This could be a reason that the author did not observe the significant P-value for some gene such as casc11.
13- As indicated in method section, author worked on 19 CML positive and 23 CML negative cases as control. In table 2, the author has been illustrated the data of all 19 CML+ cases but the data of CML – has been written as <0.1. Please describe the data of both CML+ and CML- groups in a same manner. I suggest the author to present the data as Mean±SD with Min-Max values among obtained data. This also simplifies the table2 for the readers.
14- Did the author observe any correlation between the level of BCR-ABL transcript and the expression levels of target genes PVT1, CMYC or CASC11?
15- The author found a negative correlation between PVT1-CASC11 genes in CML+ and a positive association between these two genes in CML-. How does the author explain such difference?
16- Figure 1 is not necessary.
17- The quality of figure 3 and 4 is not suitable at all.
18- The figure legends are not informative. You write a figure legend so that the reader can fully understand the content of your figure without having to refer to the main text. Please add all the necessary information. As an example, gene expression clustering should be explained in figure 3 so the readers can get the difference between red and blue signals.
19- Why did author perform the gene expression correlation through in silico analyses when he/she did it experimentally? I suggest to remove it
20- Why did the author consider the CCAT1 gene only in silico analyses and not in experimental analysis?
21- The author analyzed the miRNA-lncRNA interaction. It could be useful if the author presents the miRs with high capacity of interaction, at least.

22- I would suggest the author to re-write the discussion. It is a repetition of results. In discussion, the author should identify correlations, patterns and relationships among the data and explains the unexpected outcomes and evaluating their significance.
23- Data should be presented logically, either in the order in which the methods were presented or according to the order that most effectively develops the hypothesis. In genetics, in silico softwares facilitates the discovery of target genes and introduce potent genes for experimental projects. Accordingly, i suggest the author to move the bioinformatics section before the experimental jobs. This highlights the importance of selected genes for the readers because the author confirms the data of in silico by experimental.
Thanks.

REFEREE: Reviewer | **Revision** (0) 25 Jan 2021

Dear AE,
Comments for the authors,
The manuscript entitled" Expression and functional assessment of some featured coding and noncoding RNAs encoded by 8q24 chromosomal region in CML patients"
In this manuscript, the authors were investigated the expression and the expression correlations of MYC and two noncoding RNAs named PVT1 (circular and linear forms) and CASC11 in CML. Their data showed that noncoding genes surrounding MYC within 8q24 region might have regulatory roles in CML carcinogenesis.
Although I found it a good manuscript with interesting findings, there are some issues that the authors need to note to improve their manuscript.
Minor points:
- The English grammar and usage throughout the paper are a little poor and the paper should be revised to improve its English structure.
- Please write the ethics code in the manuscript to confirm that the ethics committee of your university has approved this research.
- The resolution of Figure 4 is not appropriate.
Major points:
- Have the patients who participated in the study received medical treatment or not? As you know and mentioned in the articles (10.1158/1541-7786.MCR-10-0356), starting treatment can affect gene expression specially MYC gene.
- The introduction is too long and it is better to write briefly about noncoding RNAs.
- Also, the discussion is long and it is the repetition of the data that should be mentioned in the results section.
- Also, the purpose of calculating the correlation coefficient is not well stated and the data related to this section are ambiguous and no useful result has been obtained.
- The introduction does not mention silico analysis and the necessity of doing this section is unclear to the readers. It is also not mentioned in the method section.
- Given that several articles have shown an increase in the expression of MYC in CML patients, why in the present study the expression of this gene does not change significantly? Could the small number of samples be the reason for this? In this case, can calculating the correlation coefficient be useful?
Thanks.

REFEREE: Associate Editor | **Revision** (0) 26 Jan 2021

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

Seyyed Ali Mard: EIC | **Revision** (0) 26 Jan 2021

Dear Author,
Your manuscript needs a minor revision in accordance with the comments made by reviewers. To avoid an extra revision, please revise it point-to-point in accordance with forwarded comments. Quick submission of the revised manuscript will guarantee a quicker final decision.
Best
EIC, Dr. Mard

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

ANSWER TO REVIERES;

Dear Dr. Ali Mard,

Thank you for giving us the opportunity to submit a revised draft of our manuscript titled "Expression and functional assessment of some featured coding and noncoding RNAs encoded from 8q24 chromosal region in CML patients" to the *Jentashapir Journal of Cellular and Molecular Biology*.

Comments from Reviewer 1;

Minor points:

We revised the texts and added ethical code for the study. Also we edited Figure 4 (presented in the revised manuscript as Figure 2).

Major points:

1-Response: The enrolled patients didn't receive any treatment. For the study we selected the samples which drawn from the cases that referred to the lab for diagnosis of CML for the first time who didn't receive any treatment related to leukemia. We added this point to the sample collection part of Method section.

2-Response: We shortened the Introduction and Discussion parts.

3-Response: *MYC*, *PVT1* and *CASC11* are located very close to each other and it's possible they shared some regulatory element in their loci vicinity. Also lncRNAs can regulate genes expression *in cis* and *in trans*. So we wanted to find out if *MYC* surrounded lncRNAs (*PVT1* and *CASC11*) have regulatory effect in cis on *MYC* using gene expression correlation analysis as it has been shown that *PVT1* has cis regulatory effects in some cancers. Although according to the presented experimental result we didn't see statistically significant concordant expression of these lncRNAs and *MYC* but *in silico* data showed they have moderate positive correlation in normal samples.

4-Response: We have done two part of insilico analysis one for gene expression correlation data (considering cis effect of lncRNA) and the other for lncRNA/miR/mRNA network. Both were done according to the possible functions of lncRNAs which we mentioned in the Introduction section. Using *in silico* analysis we wanted to shed light on some possible functional aspects of the presented lncRNAs which may present some ideas for more studies; as an instance *PVT1* and TNFα signaling pathway link in CML which we highlighted according to the *in silico* analyses results and it could be an idea for more experimental studies. We also added a short description in the Method section for *in silico* analysis.

5-Response: Limited number of samples could be a reason but we think that a much more important reason is; significant overexpression of MYC were observed in advanced phases of CML compared to healthy controls but not in . So it could be a reason for our presented result which showed non-significant overexpression of MYC (DOI: 10.1016/j.bcmd.2019.03.004 , DOI: 10.3109/10428199809057542, & DOI: 10.1016/s0145-2126(97)00006-4). It's highly possible that we have different types of CML in our enrolled samples included: blast crisis, accelerated phase, and chronic phase. We added this explanation in the Conclusion section.

Comments from Reviewer 2;

1- We rechecked and edited the manuscript text.
2- We shortened the introduction.
3- We italicized cDNA, RNA and full name of the genes.
4- Both grant ID and ethical approval number were written in the manuscript.

5- Patients' demographic and clinical presentation summarized and presented in Table 2.
6- An explanation of *in silico* analysis part was added in the Method section.
7- The data of gene expression correlation analysis and lncRNAs/miRs/mRNAs network discussed in the manuscript.
8- While BCR-ABL fusion transcripts are rarely presence in ALL an AML patients (this translocation is found in 10 to 20% of adults and in 2 to 5% of children with ALL, as well as in rare cases (1% approximately) of AML), more than 95% of CML patients representing this fused transcript as a diagnostic hallmark of the disease. Furthermore the level of BCR-ABL is lower (about 10-15%) in ALL and AML patients while in the most cases of CML the range of BCR-ABL/ABL is 30-300% with the average of 80%. Quantification of the BCR-ABL fusion transcripts in both peripheral blood mononuclear cells (PBMC) and bone marrow aspirates of patients with CML using RT-PCR has been shown to be suitable for disease diagnosis and monitoring. This description was added to the manuscript, in the Method section.
9- The expression level of genes ($2^{-\Delta Ct}$) and fold change ($2^{-\Delta\Delta Ct}$) were added in Figure1.
10- The degree of p-value added through the result section.
11- We showed MYC expression data in Result section (Figure1).
12- The reason could be correct however for p-value calculation distribution and standard deviation of the data is important. We analyzed p-value using GraphPad Prism 8 which showed significant p-value ($p<0.05$) only for linear *PVT1*.
13- The data of BCR-ABL transcript evaluation data was presented as Mean±SD in Table 2.
14- No we didn't observe any significant correlation between target genes and BCR-ABL transcript level.
15- *PVT1* and *CASC11* positively correlated in normal samples ($p=0.08$) and negatively in CML ($p=0.78$). The correlation coefficients are not statistically significant especially in CML samples which means these genes expression change in samples differently and we cannot conclude *PVT1* / *CASC11* are negatively correlated in CML. It seems that they are positively correlated in normal samples and this relation disrupts in CML which could be because of a change of their friendly interaction with enhancer elements in 8q24 to a competitive interaction its likely due to the carcinogenesis derived epigenetic modifications and subsequently inaccessibility of some enhancer element of this region.
16- Figure 1 was deleted.
17- Table 3 was added instead of Figure 3 and figure 4 edited to have more quality.
18- Figures legends were edited.
19- Because of limited experimental data we wanted to check *in silico* analyses to find out more clear data.
20- CCAT1 was removed from *in silico* data.
21- We added miR list as supplementary data.
22- The discussion part was edited.
23- Your point is completely true. But in the present study the experimental studies have not been performed to confirm *in silico* analysis data. The *in silico* section) was performed to examine a part of possible function of lncRNAs in CML in addition to experimental expression assessment.

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Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Seyyed Ali Mard: EIC | **Revision** (1) 28 Jan 2021

Dear Author,
I agreed to accept because of revising/correcting all forwarded comments in this revision.
Thanks.