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OPEN PEER REVIEW

Evaluation of the Expression Level of Some Coding and Non-coding Genes in Breast Cancer Samples Based on Bioinformatics and Laboratory Studies

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 29 Oct 2023 – 30 Oct 2023

Associate Editor

MAJOR REVISION 29 Oct 2023

Dear EIC,
My comments are:
1- The authors did not use DNase I treatment to remove DNA contamination. When the authors used GAPDH primers, DNA contamination could produce a product, causing problems in the data analysis.
2- The demographic characteristics of the patients included in the bioinformatics analysis should be explained in Table 2. For example, age, ethnicity, and so on.
3- The manuscript contains some grammatical errors. It needs to be edited by a person familiar with the English language.
4- In the methods, the authors stated that the phenol-chloroform method was used to extract RNA, whereas the phenol-chloroform method was used to extract DNA, not RNA.
5- The range of patients' ages should be added to Table 3.
6- In Figure 4, the authors should explain the meaning of *, **, and ***.
7- In the discussion, the authors should explain whether the studied genes were investigated in previous studies.

Editor-in-Chief

MAJOR REVISION 30 Oct 2023

Dear Author,
Please revise the manuscript based on the comments of the associate editor and resubmit.
Thank You

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor,

We extend our appreciation for the valuable comments and suggestions you provided regarding the shortcomings or ambiguities contained within our manuscript. We have implemented the necessary modifications accordingly, and we present a detailed breakdown of the corrections below.

Editor's Comment:

1) The authors did not use DNase I treatment to remove DNA contamination. When the authors used the primers of GAPDH, the DNA contamination could produce the product which makes the problem in the data analysis.

Our response:

The primers used in this project were specifically designed as exon junction primers, ensuring a specific connection to the desired sequences. This design helps prevent contamination with other DNA, as the primers specifically target the intended regions.

2) The demographic characteristics of the patients used in the bioinformatics analysis should be explained in the Table 2. For example, age, ethnicity, and so on.

Sample Type	No. of Samples	Gender (male/female)	Age	Age range	Vital status (Alive/ Dead)	Stage	Race
Primary Tumor	1104	13/1091 (1.18%-98/02%)	58.36	20-90	947/157(85.78%-14.22%)	I 182)16.49%) II 628) 56.89%) III 250)22.64%) IV 20)1.81%) NA 24) 2.17%)	Asian 61 (5.53%) Black 183 (16.58%) White 764 (69.19%) NA 96 (8.70%)
Solid Tissue Normal	113	1/112(0.88%-99.12 %)	57.33	20-90	69/44(61.06%-38.94%)	–	Asian 1 (0.88%) Black 6 (5.31%) White 105 (92.93%) NA 1 (0.88%)

3) The manuscript contains some grammar errors. It needs to be edited by a person familiar with English language.

The article underwent additional revisions and grammatical corrections based on recommendation from the esteemed editor.

4) In the methods, the authors expressed that phenol-chloroform method was used to extract RNA, while DNA was extracted by phenol-chloroform method not RNA.

DNA and RNA extraction from FFPE tissues was performed using the TRIzol technique, which is based on the phenol-chloroform method. We appreciate your attention to detail and the feedback you have provided.

5) The range of patient's age should be added to the Table 3.

The age range of the patients included in this research study was from a minimum of 25 years to a maximum of 78 years.

6) In the Figure 4, the authors should explain that what is the meaning of *, **, and ***.

The symbols *, **, and *** are commonly used in scientific literature to denote statistical significance levels, typically representing p-values below certain thresholds (* for p value < 0.05, ** for p value < 0.01, and *** for p value < 0.001).

7) In the discussion, the authors should explain whether the studied genes were investigated in the previous studies or not.

In the discussion section, we have referenced several previous studies that have investigated the genes examined in our research. By citing these studies, we aim to demonstrate how our study builds upon and aligns with existing knowledge in the field. Some related studies and their reference numbers are highlighted in the text.

Corrections have been made and are highlighted in green in the article. As a result, we believe that the manuscript now meets the required standards for the review process. We have resubmitted the revised manuscript to your esteemed journal and eagerly await a positive response.

2 Revision 1

2 comments · 16 Nov 2023 – 18 Nov 2023

Associate Editor

MINOR REVISION 16 Nov 2023

Dear EIC,
My comments are:
1- When we checked the GAPDH primers using the Primer blast website, the primers generated a 101 bp product with DNA as well as RNA. This led to problems in the analysis of the results.
2- The authors added an explanation about p values in Figure 4. This explanation should also be added to Figure 5.

Editor-in-Chief

MINOR REVISION 18 Nov 2023

Dear author,
Please revise the manuscript based on the comments of the associate editor and resubmit.
Thank You

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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Dear Editor,

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Editor's Comment:

1) The authors did not use DNase I treatment to remove DNA contamination. When the authors used the primers of GAPDH, the DNA contamination could produce the product which makes the problem in the data analysis.

Our response:

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2) The demographic characteristics of the patients used in the bioinformatics analysis should be explained in the Table 2. For example, age, ethnicity, and so on.

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7) In the discussion, the authors should explain whether the studied genes were investigated in the previous studies or not.

In the discussion section, we have referenced several previous studies that have investigated the genes examined in our research. By citing these studies, we aim to demonstrate how our study builds upon and aligns with existing knowledge in the field. Some related studies and their reference numbers are highlighted in the text.

Minor revision:

1) When we checked the GAPDH primers with Primer blast website, the primers generated the 101 bp product with DNA as well as RNA. This led to the problems in the results analysis.

To prevent error in the results of this project, in addition to selecting exon junction primers, DNase was also utilized during the extraction stage.

2) The authors added the explanation about p values in Figure 4. This explanation should be also added to the Figure 5.

The necessary descriptions added.

Corrections have been made and are highlighted in green in the article. As a result, we believe that the manuscript now meets the required standards for the review process. We have resubmitted the revised manuscript to your esteemed journal and eagerly await a positive response.

3 Revision 2

1 comment · 27 Jan 2024

Reza Hosseini Reviewer

REJECT 27 Jan 2024

Dear EIC, International Journal of Cancer Management

Thank you for inviting me to review the manuscript titled ": Evaluation of the expression level of some coding and non-coding genes in breast cancer samples based on bioinformatics and laboratory studies". The manuscript should be rejected.