

Peer Review File

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Reviewer A

The current study investigated the expression significance and effect of CYR61 in bladder cancer. clinical analysis and multiple in vitro cell lines were used. Results suggested CYR61 a useful diagnostic biomarker to distinguish MIBCs from NMIBCs. And functional assays confirmed it could promote MIBC cells aggressive phenotypes. This study is relatively novel and topic is interesting. However, I have several comments to their work.

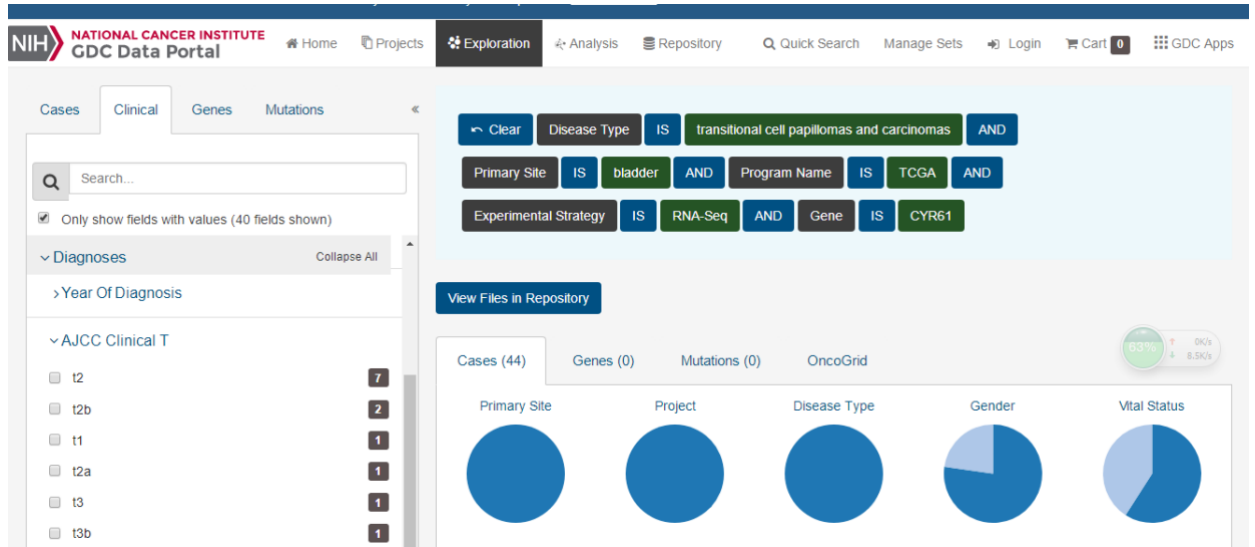
Comment 1: For survival analysis, 108 patients (from 115 cases with follow-up data) consisted NMIBC and MIBC cases. It's better to distinguish them and analysis separately.

Reply 1: Thanks for your suggestions, the relationship between CYR61 expression in NMIBC (n=56)/ MIBC (n=52) with patients' DFS/OS were separately calculated by Kaplan–Meier survival analysis. The results show, CYR61 expression have on effect on the DFS of NMIBCs ($p = 0.880$, log-rank = 0.023) and MIBC ($p = 0.292$, log-rank = 1.111); While for OS, high expression of CYR61 will decrease the OS of bladder cancer, both in NMIBC ($p = 0.043$, log-rank = 5.235) and MIBC ($p = 0.032$, log-rank = 7.213).

Changes in the text: we added a supplemental figure (Fig. S1) to show how CYR61 expression affect the NMIBC (n=56) and MIBC (n=52) patients' DFS and OS. And corresponding text was added in manuscript (see Page 15, line 287-288).

Comment 2. If possible, the authors may need to add several analyses of CYR61 expression in bladder cancer database, such as TCGA database.

Reply 2: We have investigated CYR61 expression of bladder cancer in TCGA and GEO, in these database, CYR61 was significantly increased expression in bladder cancer relative to normal tissue. While in these database, Tumor staging information of bladder cancer is incomplete, In those cases with clear tumor stage information and CYR61 gene expression, the cases classified as NMIBCs (Ta-T1) is rare (1/44, showed below), so it is hard for us to analysis of CYR61 expression between NMIBCs and MIBCs(T2-T4) in TCGA database.

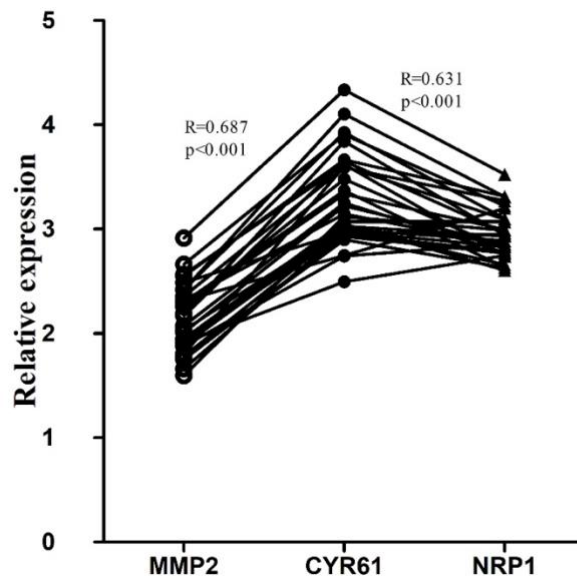


Comment 3. Please add scale bars to all microscopic images.

Reply 3: We have marked the magnification of the picture, and the scale bars have added to all microscopic images as your suggested, thanks very much for your advice. Changes in Figure 2.

Comment 4. The rationale to select MMP-2 and NRP-1 as downstream was not strong. Please clarify.

Reply 4: In the Gene expression profiling of 30 human bladder tumors we finished, we found that the expression of CYR61, MMP-2 and NRP-1 have a significant correlation. So we confirmed that in vitro. We added a supplemental figure (Fig. S4). modified our text as advised (see Page 16, line 310-313) the necessary text explaining the reason for our selecting MMP-2 and NRP-1 as downstream of CYR61.



Comment 5. Mechanism study was not detailed.

Reply 5: The purpose of this study was to demonstrate clinical value and effect of CYR61 in MIBC, so only the preliminary functional experiments have been completed in this study, and which indicating the direction for further mechanism study of CYR61.

Comment 6. Lack animal study.

Reply 6: Indeed, Animal study would provide strong evidences for CYR61 functional results got from in vitro experiments, but this study provides a direction for functional exploration of CYR61, following, we will star further study of CYR61 in the following including animal study.

Reviewer B

The authors described that CYR61 can be a potential biomarker to identify the muscle-invasive bladder cancers, even by non-invasive urine test. While the experiments were mostly well-designed, there are some questions and errors to notice.

1. The authors should check the entire manuscript for spelling, grammar and punctuation. For instance, line 104, line 191-192, etc. Please refine the use of English in the manuscript especially the discussion chapter, and ask for the services of a professional English-language editing company if possible.

Reply 1: Considering the Reviewer's suggestion, we have improved the quality of written English by AME Editing Service Company, which is the recommended language editing company by this journal.

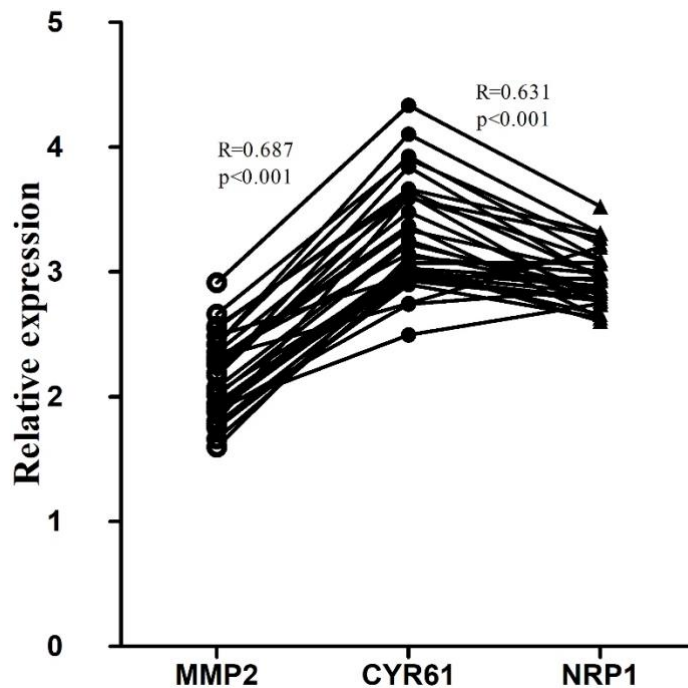
2. Some problems about the figures: In Fig 1A, it will be better to mark the point of CYR61 in the volcano plot. In Fig 4, what does 5637-pcdb mean? There were many floated cells in Fig 4B; gently washed by PBS before observation. Group descriptions or scales should be added in the figures for a better understanding.

Reply2: We are grateful for these suggestions. It had been marked the point of CYR61 in the volcano plot. In Fig 4. 5637-pcdb mean the control group transfected with the pcDNA3.1 blank plasmid. Relevant group descriptions have added in the figure's legend. Changes in Figure 1A, Figure 4, Figure 5, Figure 6. For floated cells, according to your requirements and suggestions, we repeated the scratch experiment, re-collected photos have been showed in Figure 4B.

3. In line 301, what is the function of NRP-1? Why to evaluate its expression? The authors should list the link between NRP-1 and CYR61 or bladder cancers.

Reply 3: In the Gene expression profiling of 30 human bladder tumors we finished, we found that the expression of CYR61, MMP-2 and NRP-1 have a significant correlation.

So, we confirmed that in vitro. We added a supplemental figure (Fig. S4). modified our text as advised (see Page 16, line 310-313) the necessary text explaining the reason for our selecting MMP-2 and NRP-1 as downstream of CYR61.



4. In conclusion chapter, line 410-411, the authors said this test can “get a better accuracy rate than CT or MRI”. This is not appropriate for a scientific paper without further comparison.

Reply 4: We change the related text description, Changes in text (see Page 21, line422-423). In fact, we have done related comparison work, due to the limited space of the article, it is not shown. The result of this part of work show as following:

In order to further comparison the diagnostic efficiency urinary CYR61 for MIBCs and current imageology diagnosis method. 62 in 104 bladder patients whose urine had been completed the Elisa test were got clear result of tumor stage through CT or MRI images reviewed by two radiologists. These 62 patients including 17 MIBCs and 45 NMIBCs proved by postoperative pathological stage, 11/17 MIBCs and 40/45 NMIBCs were accurately staging by CT or MRI with a sensitivity of 88.9%, specificity of 64.7% and a Youden index 0.53. urinary CYR61 (4.596 ng/mg) to distinguish MIBCs and NMIBCs, 15/17 MIBCs and 35/45 NMIBCs were accurately staging with a sensitivity of 88.2%, specificity of 77.8% and The Youden index of urinary CYR61 staging is 0.66.