Author’s response to reviews

Title: 1-Methyl-D-tryptophan activates aryl hydrocarbon receptor, a pathway associated with bladder cancer progression

Authors:

Humberto Dellè (phdelle@gmail.com)
Luiz Henrique Gomes Matheus (luiz.h.g.matheus@gmail.com)
Stephanie Vanin Dalmazzo (sthephanie.dalmazzo@gmail.com)
Rodrigo Barbosa Oliveira Brito (rodrigobarbosaoliveirabrito@gmail.com)
Lucas Alves Pereira (Lucas.alvesp1@gmail.com)
Robson José Almeida (robson.jose.almeida@gmail.com)
Cleber Pinto Camacho (camachocp@hotmail.com)

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Author’s response to reviews:

Dear Dr. Gummlich, editor-in-chief of the BMC Cancer,

We are pleased to resubmit our manuscript entitled “1-Methyl-D-tryptophan activates aryl hydrocarbon receptor, a pathway associated with bladder cancer progression”. We would like to thank you for the chance to respond to the reviewers who made constructive comments. We hope that the new version of the manuscript is acceptable for publication in BMC Cancer.

Yours sincerely,

Humberto Dellè

Comments to the Reviewers

We are very pleased to have the opportunity to read all your considerations and suggestions regarding necessary changes to our manuscript. After analyzing the proposed changes we came to some alterations that we believe to be enough to answer the risen questions and set a more accurate proposal for publication.

Reviewer 1.
Reviewer 1: The authors present a preliminary work to assess the correlation between expression of AHE and aggressive bladder cancer. The idea is innovative and could improve research of immunotherapy for bladder cancer.
I have two main concerns:
R: Thank you very much for your review. Please, find below our answers.

1. The low number of patients included for a retrospective study.
R: We agree with you. Unfortunately, there are few series in the GEO Datasets platform regarding bladder cancer. Only the GSE13507 series offered information about the stage, grade, and tumor progression. We hope that this limited number of cases does not invalidate our publication.

2. The correlation with more aggressive disease (grade and stage) is based on clinic or pathologic features? I mean did you correlate the expression of the AHR gene with TURBT stage and grade or with radical cystectomy specimens. I feel that you should implement methods to clear this point. I suggest you to refer to pathologic instead of clinic stage (I mean radical cystectomy stage and grade) to obtain better results since the stage and grade, as well disease progression is significantly different outcomes if you consider radical cystectomy instead TURBT
R: Thank you very much for the point raised. Tumors were staged and graded according to pathologic features standardized by the American Joint Committee on Cancer (AJCC). Of all 165 patients, 103 were diagnosed with non-muscle-invasive (undergoing TURBT) and 62 with muscle-invasive (undergoing cystectomy). This information was added to the text (Methods section, lines 114-124), as suggested. In addition, a new reference was added (ref 14).

Reviewer 2.
Reviewer 2: In this translational study, the authors analyzed the relative expression of aryl hydrocarbon receptor (AHR) in 165 bladder cancer patients and showed that AHR activation is associated with stage, grade and progression of BC. They also evaluated the effect of IDO1 inhibitors (i.e. 1-methyl-D-tryptophan and INCB240360) on AHR activation.
R: Thank you very much for your review. All points raised by you are really important. Please, find below our answers for each item.

Comments:
1. The manuscript requires proofreading. There are also some grammatical and writing errors that should be edited (e.g. line 35, 67, etc.).
R: Thank you very much. A careful review was made.

2. In the "background", the authors mentioned some studies, addressing the effect of AHR activation on breast cancer, melanoma, and RCC. I recommend adding more studies, focusing on the association of AHR and urothelial carcinoma
R: We agreed with this point raised by you. However, the association between AHR and urothelial tumors has been little explored so far. We have been doing careful research in the literature, and we have not found a strong foundation concerning urothelial cancer.

3. The aims of the study (primary/secondary outcomes) should be clearly defined
R: The aims were clarified at the end of the Background Section.
4. The "results" part should be reorganized; it needs more subheadings
R: The Results Section was reorganized in subheadings.

5. The actual P values should be reported rather than expressing a statement of inequality (i.e. P<.05)
R: Changes were made in the text (Results section). The p values are shown in full in Table 1.

6. Line 236,237: the authors mentioned that "In BC, AHR has been pointed as a potential inductor of carcinogenesis". This statement needs reference
R: A reference was added.

7. Again, in the "discussion", I recommend focusing on "urothelial cancer" studies rather than discussing other cancer types
R: We agree with you, however, the literature is poor on this subject. So, we built our discussion based on other types of cancer.

8. The strengths and limitations of the study should be mentioned before conclusion
R: We added a last paragraph before conclusion.

Reviewer 3.

Reviewer 3: This manuscript on the role of IDO, AHR, and CYP in the pathogenesis and the more advanced stage, grade, and progression of bladder cancer is a very well written manuscript that I enjoyed reading.
The point made by the authors is that an inhibitor of IDO1 which is 1-methyl-D-tryptophan (MT) currently studied in clinical trials is an independent stimulator of AHR, and hence despite its inhibition of the pathway, it could still result in upregulation of its downstream products. This finding, if further proven and validated, perhaps in the clinical setting in phase I trials, can be very relevant and could put an end to the trials testing for MT as treatment in bladder cancer.
R: Thank you very much for your kind analysis of our study.

Few points to suggest to the authors however:
- the introduction, last sentence of last paragraph, please fix English
R: Thank you very much. Changes and corrections were made in this paragraph.

- in methodology, please specify if bladder cancer patients had all stages from Ta to T4 with or without nodal/metastatic disease. How was progression defined?
R: All information you requested was included in “Methods” (Methods section, lines 114-124).

- in the discussion, consider adding the limitations to the study
R: We added a paragraph at the end of the Discussion section.