Author's response to reviews

Title: Chemokine mediated distribution of dendritic cell subsets in renal cell carcinoma

Authors:

Peter Middel (middel@patho-nordhessen.de)
Sven Brauneck (sven@familie-brauneck.de)
Werner Meyer (meyer@patho-nordhessen.de)
Heinz-Joachim Radzun (hradzun@med.uni-goettingen.de)

Version: 2 Date: 28 August 2010

Author's response to reviews: see over
Dear Editor,

thank you very much for considering our manuscript for revision in “BMC Cancer.”

Please find below the list of corrections. All points of criticism have been taken into account. Concerning the editorial comments:

1. A statement concerning the approval of the ethics committee of the Medical University of Göttingen as well as the compliance with the Helsinki Declaration has been included in the Methods section of the Manuscript.

2. The authors declare that they have no competing interests (included after the Conclusions in the manuscript).

3. Professional copyediting has been performed as recommended. The tracked changes of the copyediting service have been accepted by the authors. If required, a version with tracked changes of the copyedited version of the manuscript can be provided.

4. The corrections concerning the points of criticism of the referees are highlighted in the revised manuscript with tracked changes and blue letters.

Yours sincerely,

Peter Middel

List of Corrections

Referee number 1:

No points of criticism.
Referee number 2:

1. point of criticism: The study by Troy et al., The Journal of Urology, Vol. 161, Pages 1962-1967 has been cited as recommended (page 14, last paragraph, line 3; new reference 48, page 25).
2. point of criticism: The discussion has been significantly shortened for about one page and focused (page 13).
3. point of criticism: Table 1 has been updated; CCL19 has been changed to MIP-3β, CCL20 has been changed to MIP-3α, and CCL21 has been changed to SLC. The antibodies anti-CD4 and anti-Ki67 (Mib) have been included in the table 1.
4. point of criticism: Additional information concerning the species of the antibodies has also been included in table 1.
5. point of criticism: Reference 14 (page 21) was corrected. Reference number 41 has changed to the new reference number 43 and has also been corrected (page 25).
6. point of criticism: Since we found no correlation concerning tumour grading or staging and the amount of mature/immature DC infiltration in RCCs, the text passage concerning the Thoenes criteria of nuclear grading and the UICC TNM system has been removed from the Materials and Methods section (page 5, first paragraph, line 7).
7. point of criticism: Informations concerning the manufacturer of IHC Detection system has been included (page 5, second paragraph, lines 2 and 3).
8. point of criticism: Additional professional copyediting has been performed on the revised text.

Referee number 3:

Major compulsory revisions:

1. point of criticism: Figure 2 has been changed from a box-plot to a scatter-plot as recommended. As key data for each row the mean and standard Deviation have been included in the legend to figure 2.
2. point of criticism: The Mann-Whitney test with subsequent Bonferroni correction was applied the data shown in Figure 2. The test method has been included in the text (page 7, statistics section, lines 7 and 8).
3. point of criticism: MIP-3α is also expressed in lymph node tissue as shown in Figure 4 by conventional RT-PCR with gel-electrophoresis. However, the level of MIP-3α expression in lymph node tissue is much lower than in normal kidney tissue as well as RCC tumour tissue. This is caused by the fact, that epithelial cells (as well as RCC tumour cells) represent the major source of MIP-3α. In lymph nodes we found MIP-3α expression in DCs themselves as well as in reticular stromal cells by immunohistochemistry. However, the degree of MIP-3α expression seems to be by these cell populations of the lymph node much lower than tissue containing epithelia (either normal or malignant/carcinoma). For example, we found an increased expression of MIP-3α in the inflamed mucosa of colonic biopsies in Crohn’s disease, caused by MIP-3α upregulation in epithelial cells during the inflammatory process (Middel et al. 2006, Gut 55(2):220-227). In contrast, if we used total mRNA derived from tonsil tissue as a positive control for MIP-3α in real-time RT-PCR, the amount of MIP-3α amplificates was significantly higher than in total lymph node mRNA. A finding which is to our opinion caused by the presence of crypt epithelial cells within the tonsil tissue.

4. Point of criticism: The discussion has been significantly shortened and focused (see changes on page 13).

Minor essential revisions:

1. point of criticism: Reference 16 has been replaced by references 17 and 18 (page 4, first paragraph, line 5).

2. point of criticism: The sentence “Thus, questions have arisen concerning the type and activation of human DCs that should be used to induce an effective anti-tumour response” has been deleted. The role of mature DCs for the induction of a T cell immune response has been included in the Background section (page 4, first paragraph, lines 7 – 13). In addition, as mentioned by the referee, the effect of mature DCs in prevention of the development of regulatory T cells is very important; this important issue is described in the Discussion (page 15, first paragraph, lines 16 – 18).

3. point of criticism: The reference to the website of GraphPad Prism has been removed as recommended (page 9, statistics section, line 7).
4. point of criticism: Additional references concerning the expression of CD11c and Fascin by DC subsets have been included (References 19 and 20; page 8, first paragraph, lines 2 - 4).

5. point of criticism: The fluorescence stainings have been corrected in the legend to Figure 3 as recommended. The pictures are taken from different tumours which show different amounts of immune cell infiltration at the tumour border zone. Since the T cell clusters show a significant variation in their size as well as the numbers of DCs within these clusters, different densities of DCs are observed. However, the major finding is the presence of increased numbers of mature DCs in close contact to proliferating T cells. As a general rule we found that the larger the clusters of T-cells were the higher was the number of mature DCs at the tumour margin in RCC.

6. point of criticism: Additional arrows have been included in figure 3 as recommended.

7. point of criticism: Reference 52 has been changed to new reference 47 in the revised text. The finding of DC infiltration in RCC by the study of Troy et al. (1998) has been included in the text (page 14, last paragraph, lines 3 and 4; page 16, first paragraph, line 2).

8. point of criticism: Corrected as recommended (page 17, first paragraph, line 9).

9. point of criticism: Reference 14 has been updated as recommended (page 21).