Reviewer's report

Title: Increased mRNA levels of interferon alpha receptor 2 is associated with metastasis of renal cell carcinoma

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Reviewer: Bernhard Hemmerlein

Reviewer's report:

General
This aim of the present study was to demonstrate the IFN-alphaR2 mRNA is associated with tumor progress and is an indicator of IFN-alpha therapy response.
The presented experimental data do not sufficiently support this hypothesis and do not justify publication at this stage of investigation.
Furthermore, the manuscript is full of spelling errors and the used phrase are often misleading. I strongly recommend for further attempts a specific review.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
Material and Methods
Patients and tissue preparations
The authors summarize clinicopathological data in a table, e.g. tumor types, histological grade of differentiation, and TNM classification. Parts of the discussion should be summarized in this table, e.g. “In the current study, 12 patients....” (page 12, line 13ff).
The authors should further explain the term “local invasive disease”. Because every malignant tumors grows locally invasive, it is not clear, if they mean pT3a or pT3b stage in contrast to pT1 and pT2 (what I presume).

Furthermore, the authors describe that the patients received intramuscular or intravenous injections ranging between 3 and 6 million units 2 or 3 times a week. How long did the therapy last ? The dosage was dependent on the toxicity of the therapy, however, no further explanations are given which grade and type of toxicity were important. The authors do not explain what “increasing of size and number of metastatic lesions” means. It is important to know more details of the extent of distant metastasis because also the number and size of metastasis will of course interfere with prognosis and response to immunotherapy.

Reading the Material and Method section suggests that all patients were treated with IFN-a. However, in the Results section the authors describe in M0 and M1 patients a “watch-and-see” group and another hint is given in the legend of figure 1. This is confusing. Therefore, the explanation should be given in the M&M section.

The authors further analysed angiomylipoma, bladder carcinomas and testicular cancer. Renal carcinomas are malignant tumors of epithelial origin and angiomylipomas are mesenchymal tumors which behave generally benign. Did the authors analyse urothelial carcinomas or squamous or adenocarcinomas of the bladder; did the analyse G1, 2, or 3 tumors, superficial or invasive carcinomas. Testicular cancer: seminomas (which have a dense infiltrate of lymphocytes and macrophages). These additional analyses extend the study and give no relevant additional data.

Real-time RT-PCR assay
I suppose that the used primers of INF-aR2 detect all three types of mRNA splice variants. Because no gel electrophoresis was performed no data exist regarding these splice variants. This could be interesting especially because on the protein level the102 kDA IFN-aR2c variant seems to be of importance.
Another problem is that the use of a T/N ratio does not give information regarding the mRNA expression level. For example, identical ratios can be obtained with threshold cycles of 22 or 38. A clear demonstration of the threshold cycle levels in tumor and corresponding tumor-free tissues is necessary.

Western blot assay
Why didn’t the authors use a house-keeping protein such as ?-actin. This could help to estimate the amount of specific protein.
Immunohistochemistry
This section is very short and the technical citation is wrong, because the UICC classification was cited. Furthermore, it is not described how many cases were analysed. No systematic evaluation of tissue sections was performed.

Statistical analysis
I am not sure that the U test can be applied on all analyses, particularly in these cases where several rows are analysed, e.g. grade 1, 2, and 3 (see also Figure 1A). A one-way non parametric ANOVA test such as the Kruskil-Wallis test with a post test (Dunn’s) seems more appropriate.

Results
INF-aR mRNA and protein expression and pathologic characteristics
mRNA
As already mentioned the T/N ratio does not give any information regarding the mRNA expression level. This is particularly important for the interpretation of the M1 and M0 group of patients, because this could be due to high or low expression levels in corresponding tumor-free tissue (see also the IFN-aR2c protein expression in western blot analyses in M0 patients in normal tissue!). Data regarding splice variants according to the immunoblot data are necessary. Are the depicted values means and standard deviation or SEM. A description is missing. Furthermore, in line 11 page 8 a false figure is given (it should be Fig. 1B). The authors write in line 14 to 16: “... in the tumors including sarcomatoid component....conventional type...”; sarcomatoid component in clear cell carcinomas? conventional type.... is this a clear cell type or are papillary type carcinomas included? Do the authors mean M0 and M1 patients or only M1 patients? Again, the clinical-pathological data should be summarized in a table.
The part of angiomyolipomas, bladder cancer and testicular cancer should be removed (see above)
Protein
I recommend that the complete blot should be demonstrated in Fig 2 and that the proteins bands should not be “excised” artificially. What’s about IFN-aR2α?
Furthermore, as already mentioned, what is the explanation for the positive normal tissue in M0 cases in contrast to M1 cases.
Unfortunately, no data regarding alternative splicing at the transcriptional level exists. This should be added to understand the linear correlation between IFN-aR2 mRNA and aR2c protein. Did the correlation analysis include all M0 and M1 tumors?
In the immunohistochemical description only weak expression of IFN-aR2 protein was observed. However, in normal M1 tumors strong expression of IFN-aR2c can be seen; why can this not also be seen in tissue sections?

IFN-aR mRNA expression and survival
As already mentioned the T/N ratio does not allow to estimate the expression level of mRNA. The number of cases should be corrected in fig 1D: within the text 26 cases with lung metastasis are demonstrated in contrast to the figure (n=25). This can also be observed in M0 tumors: 68 cases in the text and 70 cases in fig. 1D.
To correlate mRNA expression with survival the authors used several mean ratios to discriminate between high and low expression. Although the subpopulation is different (M0+M1 and M0 versus M, resp.) the total population is identical and variable cut-off values are very problematic to use. This problem should be solved by using one cut-off value which is confirmed by the Kaplan-Meier survival rate. Comparison of Fig 1D reveals that 46 patients were treated with IFN-a and 49 patients remained untreated (n=105 ? see above). However, in Figure 4 A and B all cases were analysed together regardless of treatment. How can the authors discriminate between INF-aR2 effects based on natural IFN-a and therapeutically induced effects. I recommend to differentiate between treated an untreated cases with spontaneous course of disease.
The authors state that IFN-a and its receptor complex normally contribute to antiviral and antitumor functions. They found, that particularly a high T/N ratio of IFN-aR2 mRNA was associated with a shortened survival in comparison to tumors with a low T/N ratio and a poorer response to IFN-a treatment. This conclusion should only very carefully be drawn; as demonstrated in the results section (page 10, IFN-aR2 expression and effect of IFN-a) M0 patients with treatment which develop distant metastasis show the same T/N ratio like untreated patients. Also M0-IFN-a(+)NED patients show an increased T/N ratio in comparison to M0-IFN-a(-)/NED patients. Therefore, survival curves should discriminate between low and high IFN-aR expression and treated versus untreated patients (which was already mentioned above).

The authors further discuss that different mRNA ratios of IFN-aR1 and 2 in angiomyolipomas and renal cell
carcinomas suggest different roles of receptor subtypes (page 12, line 6ff). This is at least highly speculative and not supported by a comparison of completely different tumor entities. As already mentioned these comparison do not support the aims of this study.

Discussion
The discussion can be considerably shortened. Data which support the discussed hypothesis should be demonstrated in the Results section (page 12, line 20ff) or they shold be removed. Furthermore, the long parts of the discussion from page 13 line 9 to page 14 line 11 can be removed because they do not give answers to the central questions of the study. In summary, the discussion should be re-written on the basis of more profound experimental and statistical data.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
The authors should not use the term T/N level because it is just a ratio and does not give information regarding the level of gene expression

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Discretionary Revisions (which the author can choose to ignore)

What next?: Reject because scientifically unsound

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes

Declaration of competing interests:
I declare that I have no competing interests