Reviewer's report

Title: GLUT1 gene is a potential hypoxic and prognostic marker in colorectal cancer patients

Version: 2 Date: 30 December 2008

Reviewer: Lothar Fecker

Reviewer's report:

Comments from Lothar F. Fecker to answers of authors (1. Review process)
Date: 29.12.2008
Title:
Glut1 gene is a potential hypoxic and prognostic marker in colorectal cancer patients
Authors:
Fu-Yen Chung Ming-Yii Huang, Ching-Sheng Yeh, Tian-Lu Cheng, Li-Chen Yen, Jaw-Yuan Wang, Shiu-Ru Lin

In the above mentioned manuscript Fu-Yen Chung et al. report their data on expression of hypoxia-related and glycolysis-related genes in colorectal cancer tissues and colorectal adenocarcinoma cell lines under normoxic and hypoxic conditions. As hypoxia is a characteristic feature of aggressive tumors, the authors investigated whether typical marker genes for hypoxia (GLUT1, HIF-1#, HIF-2#) can be identified in colorectal cancer patients, which may be applicable for diagnosis, prognosis and monitoring of recurrence after treatment.

Comment:
The first revision has been done carefully by the authors and my comments which require major revisions or minor essential revisions have been answered well. Appropriate changes have been done and additional explanations were given in the manuscript.

I recommend the above mentioned manuscript for publication after few corrections have been done and few additional explanations are given in the text.

-Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

none

-Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Page 3; Abstract; lanes 15-16:
I think you should exchange SW480 (...) for (SW620 (...))
2. Page 7; lane 11:
please write: … to investigate the correlation of hypoxia-related genes …

3. Page 9; Chapter: Reverse transcription-polymerase chain reaction (RT-PCR)
I suggest to alter the order of the first sentences in the chapter in this way:
RNA was extracted from colorectal cancer cell lines using ……….Total RNA (2µg) was reverse transcribed to cDNA and two microliters of each of these cDNA samples were used for each PCR reaction.

4. page 12; lanes 7-13:
I think you can omit the sentence beginning with “Siteen tartget genes (..)…” as this you have also written in the legend to figure 5.” But if you do so, please mention in the following sentence that the housekeeping gene was #-actin and the bacterial gene was from Mycobacterium tuberculosis!

5. page 13; lane 2:
Please describe procedure of hybridization, incubation with antibodies and especially the blocking step (when? After incubation with antibodies??) more precisely.

6. page 19, lanes 19-21:
Please check the grammar of this newly introduced sentence. I don’t understand what you mean.

7. page 21; lane 21:
you should make a correction here: ..from early stages through advanced stages of...

8. Legend to figure 2:
a. Please describe shortly what was the control
b. Please describe how the relative expression ratio was calculated. It should be clear for the reader whether the measured values in a series were normalized to the #-actin signal of the respective control or whether each measured value was normalized to the corresponding (individual) #-actin signal.

9. Description of Table 4
Also here you should describe more precisely how the ratio of gene expression was determined:
Was it (??): Gene expression ratio of colorectal cancer tissues (normalized with corresponding #-actin signal) compared with gene expression ratio of paired normal colorectal tumor tissues (normalized with corresponding #-actin signal)

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore):
None

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests