Reviewer's report

Title: Alteration of protein expression pattern of vascular endothelial growth factor (VEGF) from soluble to cell-associated isoform during tumourigenesis

Version: 1 Date: 8 July 2005

Reviewer: Rainer Broll

Reviewer's report:

General
The authors claim in the title that there is an alteration of protein expression pattern of VEGF from soluble to cell-associated isoform during tumourigenesis. I can not duplicate this theory by reading the data of the study. They demonstrated only a significant change in VEGF isoform 189 (26 kDa) expression from early to late tumor stage in colorectal cancer and also but not significant in lung cancer. Besides this they found that VEGF 121 is more present in smaller tumors (<5 cm) than in tumors > 5 cm. This is not sufficient to prove their hypothesis. The second question, whether there is a relationship between the expression pattern of VEGF isoforms and the level of the circulating VEGF in serum could not be answered, and they did not find any correlations to pathological features nor to VEGF isoforms. Furthermore, it is important to consider that authors used an ELISA assay which recognizes only VEGF165 but not VEGF 121 and 189 in serum. For this reason a direct comparison between VEGF protein expression in tumor samples and VEGF serum levels is not useful. Regarding these facts the title indicates something which is not really proved in the study. Furthermore, authors "assume/believe" that protein bands with molecular weight of 18 and 26 kDa correspond to VEGF121 and VEGF189. They have to prove it as it was done with the 23 kDa protein (VEGF165).

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

Both conclusions (abstract and paper) have to be changed (see general).

Part "Background":
Last paragraph: citation [7] is wrong; the citated paper deals with non-small cell lung cancer and not with colorectal cancer.

Part "Methods - Selection of patients and sample":
There is no information about the percentage of tumor tissue in a tumor sample. However, this is of importance, because this can have an affect on the VEGF expression profile. Therefore it must be at least 80%.
There is no information in the paper, why only sera from 56 cancer patients were examined and not from all 94 patients.
We know that recent trauma, surgery, pregnancy or diseases like rheumatoid arthritis elevates the VEGF serum level. Was this excluded in the group of healthy volunteers? Were both groups (tumor patients and volunteers) comparable regarding age and gender? Data should be demonstrated.

Part "Western blotting":
Detailed information about the VEGF-antibodies used in the western blot and also their dilution has to be demonstrated.
Part "Legends":
"Figure 3" is in reality "Table 3"!
"Figure 2" is not cited in the text!

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

A few mistakes in the text should be eliminated: e.g.

Part "Background":
last but one line: demonstrated = demonstrated

Part "Western blotting":
Line 12: ant-VEGF = anti-VEGF

Part "Results - Levels of circulating VEGF..."
Line 10: believed = believed

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No