Reviewer's report

Title: Interstitial fluid pressure, vascularity and metastasis in ectopic, orthotopic and spontaneous tumors.

Version: 2 Date: 10 June 2007

Reviewer: Professor Rubin

Reviewer's report:

General

This manuscript describes a thorough study of interstitial fluid pressure (IFP) in experimental carcinoma models. A major part of the manuscript deals with a comparison of tumor IFP in a set of tumor models grown at various implantation sites. Tumors implanted intra-muscularly (i.m.) displayed significantly higher IFP than tumors implanted sub-cutaneously (s.c) or at an orthotopic site (cervix). The spreading between recorded IFP values grown at the same location and within the same tumor type was, however, limited. This was the case for 5 different investigated tumor models. The authors also aimed to investigate correlations between IFP on the one hand, and metastases, lymph vessels, hypoxia and blood perfusion on the other. Basically no correlations were found.

A major criticism of the manuscript is that it is hard to get bottom line of the study. The conclusion section covers more than a page. No specific mechanistic insights are provided by the present collection of data. The general conclusion that tumor IFP varies with tumor type and implantation site is not novel and this conclusion can be drawn from published data. Also, the presented data show that IFP varied remarkably little between various tumor types (means between 6 and 9 mmHg with modest spreading within each cohort of the different tumor types) with the exception of SiHa tumors grown orthotopically with a mean IFP of 14 mmHg. The largest differences were seen when tumors were grown i.m. compared with growth at the other sites, however, no experimentally validated conclusions can explain this difference. The finding of a potential correlation between blood perfusion and IFP is of interest but is not experimentally followed up. The data on a lack of relation between metastases and IFP is also interesting.

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

The text is at places hard to follow and vague. Examples are listed below.
1) Abstract, conclusion (line 4). What is meant by '...irrespective of previous molecular interactions was further...' Previous to what? Which molecular interactions?
2) Abstract, last sentence. What is meant by '....future studies may be more effective using tumors grown orthotopically,...'. Effective in what sense? Whether orthotopic or e.g. sub-cutaneous (s.c.) models should be used has to be determined by taking into account the aim of the experiment, availability of technology and ethical considerations (animal welfare). It is obvious that in general terms orthotopic growth more closely mimic a clinical tumor, but for specific studies may not be the most effective choice. Also, in the models investigated the differences in IFP between cohorts of tumors grown sub-cutaneously or orthotopically were similar.
3) Introduction, first sentence. It is stated that the elevated tumor IFP largely is due the abnormal vasculature generated via angiogenesis. This statement raises two questions. First, which mechanism other than angiogenesis forms a vasculature in tumors? Second, in the further text other mechanisms for the elevated tumor IFP are given. Thus, in the Discussion (page 17, line 11) it is stated that the stroma (collagen and fibroblasts) together with infiltrating macrophages act to elevate IFP. On page 18, line 10 it is stated that the elevated tumor IFP most probably is a result of the combination of genetics, the mechanics at the recipient site and host-tumor physiologic interactions. Finally, in the Conclusion (page 20, line 11 from bottom) it is stated that the present data suggest 'that factors influencing tumor IFP are complex and in need of further study'.
4) Page 18, line 10. The sentence is vague. Which genetics? Do tumor cells have physiologic interactions with the host?
5) Conclusion, page 19 onwards. The text rambles and it is hard to understand which are the major conclusion(s) of the study.

The Introduction and Discussion sections should be rewritten in a more stringent and coherent way. In the Discussion it should be stated which of the mechanistic possibilities for an increased IFP that is favored by
the present data and discuss this in the context of previous reports.

Key experiments are not well described.
1) Fig 5. Lymph vessel density (LYVE-1), hypoxia (EF-5) and PECAM distributions are shown in low-power fields from ME180 xenografts. Sections were compared by pixel-counting on whole tissue segments, which show low variations between the different tumor groups and locations. Data on spatial distributions would be of more interesting and needed in order to draw the conclusion of that there is no correlation between IFP and the studied parameters. Better data as to these relations would be needed.
2) Fig 6. What is meant by, 'the maximal perfused area for each tumor of the 5 planes analyzed'. Is the plane with the highest perfused area selected or what? Is it suggested that the modest difference (Fig 6D) could in part explain the rather large difference in IFP between ME180 tumors xenograft i.m. or orthotopically. Is there no difference in perfusion between tumors grown s.c. and orthotopically? A potential relation between perfusion and IFP is interesting and these data should be expanded. Three samples in each experimental group is too small.
3) I suppose that metastases were assessed in animals bearing primary tumors in which IFP had been determined. Perhaps it would better to plot IFP versus metastases for each animal rather than dichotomize in high and low pressure.

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

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, but I do not feel adequately qualified to assess the statistics.

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