Reviewer's report

Title: Matrix gla protein (MGP): an overexpressed and migration-promoting mesenchymal component in glioblastoma

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Reviewer: Dr. Carsten Hagemann

Reviewer's report:

The manuscript "Matrix Gla protein (MGP): an overexpressed and migration-promoting mesenchymal component in glioblastoma" reports overexpression of MGP by glioblastomas on mRNA and protein level in comparison to normal brain tissue. In addition, it addresses the function of this protein by showing its involvement in the regulation of migration of diverse glioblastoma cell lines in cell culture experiments using RNA interference. The data are sound, well presented and of interest in its field. Therefore, I support publication by BMC Cancer after some minor revision.

- Minor Essential Revision

1. The authors show a quite large variation of MGP mRNA expression if biopsies from different patients are compared (Fig. 1). Therefore, I would like to see some data concerning the tumor samples used. Were these primary diagnosed glioblastomas or has recurrent disease been included into the study? Are the tissues from primary glioblastomas or were also secondary glioblastomas which developed from astrocytomas of lower grade included? Are there differences in MGP expression between these groups? What about survival data? I am aware that the sample size is too small for statistically significant statements, however, is there a tendency that patients with low MGP expression show a longer survival compared to those with high expression in this study? Is there any influence of location of the tumor in the brain, age or gender of the patient and the expression of MGP?

2. Was immunohistochemistry done with the same specimen used for PCR-experiments? Is there correlation between mRNA level and strength of protein expression in the tumor samples?

3. The cell lines showed different level of MGP mRNA expression (Fig. 1). The authors state that "these findings were confirmed at protein levels". However, data from ELISA analysis are only given for U373fast and U373slow cells. I would suggest to include a table showing the ELISA results for all cell lines analysed, since it does not seem to be possible to see differences in MGP expression strength by immunohistochemistry (Fig. 2: H4 cells - weakest in PCR display the same expression strength as U373fast and U343MG, respectively). Alternatively, a Western-blot showing MGP expression in all cell lines used in comparison to a housekeeping protein could be presented.
4. Figure 1:
I would change the sequence of the different sample groups. The figure should start with normal brain in the first lane, since this is the control, followed by GBM in the middle and the cell lines in the last lane. This orientation would match the text and the order of samples shown in other figures.

5. Language
I am not a native speaker either. However, I found some spelling errors and would use a slightly different phrasing from time to time:

Throughout the text authors should decide whether they want to place a space between figures and units or not. Formatting should be uniformly.

page 4: "Matrix Gla protein" is written with a capital. Throughout the text "gla" is written in lowercase.

page 5: References [12-16] are cited in superscript.

page 6 - cell culture: rephrase to "... using standard cell culture conditions in Dulbecco’s Modified Eagle’s minimal essential medium (DMEM) supplemented with ...

page 7 - immunohistochemistry: "... 10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween ...

page 7 - ELISA assay: "... DMEM without FCS for 24 h (1 x 106 cells)."

page 9: "All experiments were done independently for at least three times."

page 12: "... 1.1%-12.9% at 24 h ..."; "...(72 h post-transfection) revealed decreased migration..."

page 14: "in situ" should be in italics

page 20 - Figure 3: "... as determined by quantitative RT-PCR. MGP mRNA is expressed relative to control transfections ... on protein levels by immunofluorescence staining ...

page 20 - Figure 4: "... following MGP knockdown in a wound-healing assay."

page 21 - Figure 4: "All differences between MGP siRNA and cells transfected with control siRNA were significant (p < 0.01)."; "... as compared to the control cells, transfected with non-silencing siRNA ...

page 21 - Figure 5: "... as compared to cells transfected with non-silencing siRNA ...

- Discretionary Revision
> These are recommendations for improvement which the author can choose
to ignore. For example clarifications, data that would be useful but not essential.

The impact of the paper could be increased by analysing MGP expression also in astrocytomas WHO grade II and III. Is there a gradual increase in MGP expression concomitantly with increasing malignancy?

Is it possible to overexpress MGP in glioblastoma cells? By doing so, is it possible to speed up migration of slowly migrating cells? Is it possible to rescue the knock-down phenotype by overexpression of an MGP construct insensitive to the siRNA used?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.