Reviewer's report

Title: Involvement of extracellular ATP on the glioblastoma growth in a rat glioma model.

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Reviewer: Rafal Czajkowski

Reviewer's report:

General

The authors of the manuscript â€” Involvement of extracellular ATP on the glioblastoma growth in a rat glioma modelâ€” have undertaken an interesting and creative approach towards establishing the role of ATP as a signalling molecule in the regulation of tumor growth. This experimental model might be useful not only in the studies of ATP hydrolysis, but might provide a framework for a more elaborate screening of nucleotide signalling in tumors. The specific results presented in the manuscript, however, are not acceptable because of a possibility of a serious flaw in methodology.

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

The results presented in the manuscript figures seem reasonable, significant and very striking. However, it has not been explained how a single, acute injection of an active enzyme would provide sufficient apyrase activity for prolonged period (20 days). It is generally accepted that the activity of extracellular proteases is relatively high in tumors (the authors even mention this fact in the manuscript, p. 4, line 12 and subsequent citation). Therefore it is very unlikely that the enzymatic activity of the injected apyrase would persist for more than few hours. The observed phenomenon could be explained by a more reasonable mechanism. After harvesting, C6 cells are collected and handled in DMEM without growth factors until the injection. Under such stressful conditions (lack of both adhesion and serum), ATP might act in an auto/paracrine manner as the â€œlast resortâ€ growth factor that prevents cell death. Therefore at the very moment of the inoculation, a significant fraction of the glioma cells treated with apyrase might be already unable to form the tumor. This possibility is fully justified by the previous results obtained by the authors (p. 5 lines 3-8) and other groups. The control experiment performed by the authors (p. 12, lines 15-17) does not reproduce the experimental conditions because the glioma cells are not detached form the surface. These issues have to be addressed experimentally in order to make the results reliable.

First, the amount of viable glioma cells in the brain after injection needs to be confirmed. The suggested time points are 24 and 48 h post injection. The standard tests for apoptosis and proliferation would certainly be convincing. Also, a simple (but slightly less convincing) method to perform this control would be to re-plate the cells handled exactly as for injection and to compare their survival rates after 24 and 48 h in DMEM/FCS.

Second, the activity of apyrase in the tumor tissue has to be monitored during the experiment. At least several crucial time points (1 hour, 1 day, 7 days and 20 days) are necessary. After collection of the tissue, the presence of the intact protein (western blot) or enzymatic activity (hopefully this is possible in such a small sample) should be detected.

If the results of the mentioned control experiments are positive (i.e. the initial number of viable cells is indeed lower, and/or the apyrase activity is decaying), the whole issue should be readdressed. There are several ways to tackle the problem.

- A molecular approach: to enhance the expression of the apyrase in glioma cells (preferably in an inducible manner) and compare tumors with induced and non-induced enzyme expression.
- A surgical approach: to provide constant supply of apyrase to the forming tumor, for example by using the osmotic pump, starting no earlier than 48 h after inoculation.

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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 manuscript is well written, but the title could be more specific. Since ATP is studied only indirectly, mentioning apyrase in the title would be more appropriate.
The introduction and discussion are generally well balanced, with one exception. On page 5, par. 3 (the hypothesis), lines 15-16, the authors hypothesise that ATP might act by inducing cell death of surrounding normal tissue and creating more space for growing tumor. This particular possibility is not tested in the subsequent sections (no test for apoptosis in surrounding tissue, just within the tumor itself) and therefore should be moved to the Discussion.

Discretionary Revisions (which the author can choose to ignore)

None

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 importance in its field

Quality of written English: Acceptable

Statistical review: No

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