Author's response to reviews

Title: Nestin expression in the cell lines derived from glioblastoma multiforme.

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Author's response to reviews: see over
Dear Editors,

We would like to submit a second revised version of our manuscript entitled “Nestin expression in the cell lines derived from glioblastoma multiforme” by R. Veselska, P. Kuglik, P. Cejpek, H. Svachova, J. Neradil, T. Loja and J. Relichova.

In the text below, we explain all changes made according to the reviewer’s suggestions. For easier evaluation of this revised version of manuscript, all changes recommended by reviewers are typed in blue.

As suggested by Reviewer Prof. Ross, this version of manuscript was also copyedited by native speaker.

Sincerely,

Dr. Renata Veselska

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REVIEWER: ROBERT A ROSS

Major Compulsory Revisions (that 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. Since the karyotypes have been removed from the paper, the section in “Methods” (page 7) on “Karyotyping” and the preceding sentence concerning colchicine treatment should likewise be deleted.

   The section „Karyotyping“ was deleted, as well as the last sentence concerning colchicine treatment in preceding section.

2. The new paragraph at the beginning of the Results section, changed in response to both reviewers’ comments, is perhaps too brief. The sentence about GM7 is fine, but slightly more information is necessary to prove the tumor origin of GM10: to say that it is “near-diploid” is not enough. Add back the number of gain/lost chromosomes and of marker chromosomes? Also, if you did FISH, CGH, and HR-CGH, why not mention that in the text or omit it from the manuscript?

   The results of GM10 cell line karyotyping were added back, as reviewer suggested. Moreover, required remark concerning performance of FISH and HR-CGH was added in this paragraph.

The GM10 cell line was described as being near-diploid. The number of chromosomes varied from 43 to 46 and chromosomes 3, 15, 19, 22 and Y were the ones most frequently found to be missing. Genetic changes in both cell lines were also confirmed using FISH and HR-CGH methods (data not shown).
3. The new results section, page 10, last sentence, top paragraph states that “a portion of nestin-positive cells was progressively reduced up to complete . . . . This is very confusing English and must be modified.

The sentence was modified and subdivided:

During short-term cell cultures (between passages 2 and 4), a decreasing number of nestin-negative cells were observed. At passage 5, all cells in the population showed nestin positivity.

4. The description for E in Figure 8 legend should be labeled F in the legend.

Mistaken labeling in Figure 8 legend was corrected:

Nestin was detected using immunogold labeling; bars, 1 µm (A), 0.1 µm (B-E). Negative control stained with secondary antibody only: nucleus and nucleolus (Nu); bar, 0.5 µm (F).

Discretionary Revisions (which the author can choose to ignore)

1. The cells chosen for illustration for the software cross-section and co-location studies, showing nestin in the nucleus, all have the more wide-spread pattern of nestin filaments throughout the cytoplasm. Do cells with the smaller, discrete arrangement of nestin (as in Fig. 2C) also show nuclear nestin? If yes, is the nestin more frequently found in filamentous structures or spirals? Is there any difference in co-location with MTOC and/or microtubules? In other words, how general is this phenomenon.

Nuclear nestin was detectable also in smaller cells using epifluorescence and confocal microscopy. Therefore, this phenomenon seems to be general in our cell lines and also the results of double labeling of nestin and tubulin brought no difference in these cells. Most of cells examined using transmission electron microscopy and ultrathin sections also showed positivity for nuclear nestin. However, it is difficult to specify the type of these cells, because the cell shape in section depends on cell position and orientation in the embedding medium.

2. This manuscript, and in particular the discussion section, requires editing for grammar and syntax.

The revised manuscript was copyedited by native speaker.

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

No revision is required.

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

One typographical error:
Page 5, line 6; [showed] should be [shown].

The error was corrected:

...nestin has also been shown...

Addition:
Figure legend for Figure 8C:
Missed what an arrow indicates. An arrow indicates very short fibers.

The description was added:

Arrows indicate very short fibers in these aggregates (B-C).