Reviewer's report

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

Version: 2 Date: 25 July 2005

Reviewer: Robert A Ross

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General
The manuscript entitled “Nestin expression in the cell lines derived from glioblastoma multiforme” by Drs. Veselska et al. describes immunocytochemical and immunoelectron microscopic studies examining the localization of the intermediate filament nestin in primary cell cultures and cell lines from two glioblastoma tumors. The authors attempt to show that nestin expression varies with cell phenotype and mitosis and does not co-localize with MTOC and microtubular networks. More importantly, the authors provide evidence for localization of nestin within the cell nucleus. The data are generally convincing and of marked interest. However, the paper is not well focused, reducing the enthusiasm of this reviewer.

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

1. It is not clear whether the authors are attempting to correlate nestin cytoskeletal arrangement, being diffuse or in a traditional filamentous network, with cell morphology and/or transformed phenotype. This needs to be clarified. Along the same lines, the legend to Figure 1C describes “non-tumor cells exhibiting no or very poor signals for nestin”, whereas the text implies it is the tumor cells that differed widely in amount of nestin.

2. The co-localization studies with nestin and tubulin are not convincing. Whereas nestin does not appear to co-localize with the MTOC (as described by others), the asymmetric distribution of both the microtubular and nestin networks (shown in Fig. 4) do not appear to be random and suggest interaction. Nestin appears to be distributed throughout the cell rather than localized to one area, and it is impossible to determine the position of nucleus and the edge of the cytoplasm to orient oneself. Inclusion of a DAPI stain or of a phase contrast illustration would be helpful. Also, it would be helpful to indicate the nucleoli, mentioned in the text, by arrows on Figure 5.

3. The authors’ suggestion – that nuclear staining of nestin is seen in Fig. 4 – is difficult to see and harder to interpret. Since these are whole cells, diffuse staining of nestin filaments above or below the nucleus could be perceived as intranuclear. While the authors state that there is nucleolar staining (possibly seen as the punctate green stain in Fig. 5), they are neither clear on this point nor do they illustrate it in their confocal micrographic analysis or their EM photomicrographs.

4. The authors do not propose a function for the nuclear localization of nestin nor do they correlate it with malignancy. Thus, the authors need to speculate about the function of nuclear (and possible nucleolar) nestin.

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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)

1. It would appear from the Abstract that this would be a paper detailing the role of intermediate filaments, and especially nestin, in tumor malignancy – a topic not addressed at all in the Results section. Instead, the paper is essentially a description of the organization of nestin in glioma cells, in culture, during interphase and mitosis. Interesting, but not what is expected.

2. Inclusion of cytogenetic analyses is irrelevant to the main thrust of their study and could be omitted. However, if it is to be included, there should be an actual karyotype to allow the reader to
judge the quality yielded by these cells. Moreover, according to ISCN 1995, the description should be expressed relative to the closest ploidy (in the case of GM7, to a triploid or tetraploid chromosome number).

3. Given the substantial normal diploid population in GM10, what were the percentages of cells that were positive for vimentin and/or GFAP? One long time observation is that growth in culture frequently selects for vimentin-expressing cells, and normal fibroblasts also express vimentin. Why not provide an illustration?

4. It would be helpful to the reader for the photomicrographs of nuclear staining images (Fig. 7 C, D and Fig. 8C) to be in the same figure, separate from those with cytoplasmic staining. Also unclear is what the authors mean by “short fibers” when referring to nuclear staining. Is this referring to the linear arrangement of immunogold stain, which could be binding to strands of DNA, and not filamentous nestin? Why is the negative nucleolar stain included in Figure 8 rather than 9?

5. There is a typographical error in the legend to Figure 9 “(B-X)” should be “(B-C)”.

Discretionary Revisions (which the author can choose to ignore)

1. Figures 2 and 3, although quite pretty, show essentially the same thing and should be combined and condensed. More informative, since there is some variation in pattern of nestin staining would be inclusion of photographs showing dual labeling with vimentin and nestin or GFAP and nestin, to see if they do co-localize.

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'