Reviewer’s report

Title: Protein p16INK4a as a marker of dysplastic and neoplastic alterations in cervical epithelial cells

Version: 1 Date: 21 April 2004

Reviewer: Karin Milde-Langosch

Reviewer’s report:

General
Although the incidence of invasive cervical cancer has decreased since the introduction of Pap tests as a screening method in developed countries, there is still a proportion of false-negative or false-positive results. Therefore, additional markers for cervical neoplastic lesions might be of general interest. In their study, Volgareva et al. analyzed the expression of the cell-cycle inhibitor p16/Ink4a, which was shown before to be up-regulated in most cervical cancers showing expression of the HPV oncogene E7, in various cervical lesions. Although this is not the first report on this subject, the large number of 194 cervical samples makes it a valuable study which deserves publication after revision.

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

1. Methods, chapter 5: The processing of vaginal smears is described in this section. Yet, it is not clearly described which of the analyzed samples were vaginal smears and which samples were paraffin –embedded tissues. This information must be included in the text and in table 1. In addition, the authors should give some information about the quality of the results in cytological versus histological specimens.

2. It is not clear if HPV detection by PCR was performed in all samples, in CIN and carcinoma samples or only in a fraction of the cases. The correlation of p16 results with HPV status (negative / HPV 16 / HPV 18) should be presented clearly in the results section, i.e. as an additional table.

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

3. The term "endocervicoses" is unusual and not clear. The authors should explain what it means in histological diagnosis.

4. The subcellular location of the positive staining (nuclear/cytoplasmic) should be described in the results section, not only in the discussion.

5. On page 6, the authors discuss that the antibody E6H4 has some advantages compared with other antibodies which were tested in their lab. These comparative experiments should be described in more detail including the names of the other tested antibodies in the results section, or this chapter should be omitted. Since the authors probably did not test all commercially available p16-antibodies, the term "... the most p16-specific monoclonal antibody" (abstract, page 2) should be replaced by "... a highly p16-specific antibody".

6. In Fig. 1, only negative and diffuse staining is shown in several examples. It would be useful to show an example of sporadic or focal staining, too.

7. In the last sentence of the discussion, the authors state that endometrial cells express p16INK4a. This is in contrast to the negative result obtained with one sample from the uterus (fig. 2) and should be further explained.
8. Methods: positive and negative controls for immunohistochemistry should be described.

Discretionary Revisions (which the author can choose to ignore)

9. Page 3, chapter 4: "To detect high risk HPV's..l.": PCR has not been developed for HPV detection but applied to this purpose.
10. Page 3, chapter 5: "p16INK4a expression ceases under such condition...". P16 expression does not always cease. It might also be reduced (i.e. after LOH), or protein function might be impaired by mutations.
11. Tables 1 and 2 might be combined.

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: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

None