Reviewer's report

Title: Up-regulation of expression and lack of 5CpG island hypermethylation of p16 INK4a in HPV-positive cervical carcinomas.

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Reviewer: Werner Zwberschke

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General / Minor Essential Revisions

Review Ivanova et al., 2006:
Although the cyclin dependent kinase-inhibitor p16INK4a can act as a tumour suppressor protein high levels of p16INK4a are frequently detected in human papillomavirus (HPV)-positive cervical squamous cell carcinomas. Since the retinoblastoma protein (pRb) is a negative regulator of p16INK4a gene expression, the inactivation of pRb by the high risk HPV E7 oncoprotein leads to derepression of p16INK4a transcription, and consequently, p16INK4a will be expressed at high levels, as is found in high-risk HPV-positive cervical cancers. In contrast, in many virus-negative squamous cell carcinomas, the p16INK4a/cyclin D/cdk4,6/pRb pathway is frequently inactivated through mutation, and/or hypermethylation of the p16INK4a gene, resulting in reduced or absent p16INK4a expression.

To address the mechanisms underlying p16INK4a gene expression in cervical cancers the authors analysed the methylation status of the p16INK4a promoter region in HPV-16 and -18-positive cervical squamous cell carcinomas and in cervical cancer cell lines using bisulphite-modified DNA sequencing. Moreover, the HPV-E7 oncogene expression and the p16INK4a mRNA and protein levels were monitored in the same specimens. It is demonstrated that the 5'CpG-rich sequences in the p16INK4a promoter are neither hypermethylated in 13 out of 13 primary cervical cancers nor in 5 out of 5 cervical cancer cell lines. This is consistent with the findings, that the p16INK4a mRNA and protein levels are increased in the cervical cancer specimens relative to normal tissues. In accordance with previous studies high levels of the p16INK4a protein were detected in the cytoplasm and the nucleus of the cervical cancer cells. Moreover, the authors showed that the high p16INK4a protein levels are strongly associated with the expression of the HPV-E7 oncogene.

The importance of p16INK4a gene regulation in cervical carcinogenesis is an active area of HPV-research at present. The present study by Ivanova et al. is interesting and adds to the better understanding of the mechanisms underlying the control of p16INK4a expression in cervical cancer. While this work suggests that high p16INK4a expression is associated with low level p16INK4a promoter 5'CpG methylation in HPV-E7 expressing cervical cancers, previous studies showed significant 5'CpG methylation of the p16INK4a promoter in a certain proportion of primary cervical cancers and cervical cancer cell lines. However, in these studies, the p16INK4a gene expression was not always analysed to define the significance of the p16 promoter methylation and gene expression or the found 5'CpG methylation of the p16 INK4a promoter correlated not well with downregualtion of p16INK4a expression. Furthermore, the HPV-E7 mRNA expression was not always determined.

The study by Ivanova et al. suggests that inactivation of pRb by the E7 oncoprotein contributes to upregulation of p16 Ink4a gene expression in cervical cancer cells, and hypermethylation and downregualtion of the p16 INK4a gene might not be critical in those cells to override of the p16 INK4a/pRb cell cycle check point. The methods are appropriate and well described and the results are sound and well controlled. The abstract and title do accurately convey what has been found. I have no major points of criticism. Reference 27 is not correct cited. A few spelling mistakes should be corrected. I recommend this study for publishing in BMC Medicine.

What next?: Accept after minor essential 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, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.