学 位 論 文 の 要 旨

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学　位　論　文　名　An Evaluation of the Diagnostic and Prognostic Significance of $p16^{INK4a}$ / $p21^{WAF1/CIP1}$ Immunostaining in Squamous Intraepithelial Lesions of the Uterine Cervix Using Liquid-Based Cytology Specimens

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論 文 内 容 の 要 旨

INTRODUCTION

Human papillomavirus (HPV) infection causes squamous intraepithelial lesions (SIL) of the uterine cervix、which frequently progresses into squamous cell carcinoma. It is therefore important to identify cases that potentially develop higher grades of SIL at an early stage of the disease. Although cytological diagnosis has been widely used as a screening method of SIL and cancer of the uterine cervix, such prediction is so far difficult practically.

The $p16^{INK4a}$ (p16) protein is a cyclin dependent kinase (CDK) inhibitor that decelerates the cell cycle by inactivating the CDKs. Overexpression of p16 has been demonstrated in SIL and squamous cell carcinoma with HPV infection. Thus, the positive p16 immunostaining in cervical epithelium is used as a surrogate marker of HPV infection.

Another CDK inhibitor, $p21^{WAF1/Cip1}$ (p21) is induced by the activation of p53, and is known to play important roles in anti-oncogenesis. Although this CDK inhibitor is therefore expected to be another useful marker for SIL, applicability of p21 in the cytological diagnosis has not been fully evaluated yet. In this study, we thus investigated whether immunocytochemistry for p16 and p21 could be applicable in the diagnosis and the prognostic prediction of SIL in combination with genomic analyses of HPV.

MATERIALS AND METHODS

A hundred and forty-nine cytological samples were collected from consecutive patients who newly visited the Department of Obstetrics and Gynecology of the National Hospital Organization (NHO) Hamada Medical Center between May, 2008 and March, 2011. After smear samples were prepared, the brush was washed in Thinprep PreservCyt Solution to recover and fix the residual cells. These cells were then used in liquid-based cytology preparation for immunocytochemistry for p16 and p21 as well as for in-situ hybridization (ISH) and the reversed dot blotting for HPV genotyping. Cytological diagnosis was made on smear specimens according to the Bethesda system. We found 76 cases of 'negative for intraepithelial lesion or malignancy (NILM)', 28 of 'low-grade SIL (LSIL)', 30 of 'high-grade SIL (HSIL)', and 11 of 'atypical squamous cells of undetermined significance (ASC-US)'. Multiple logistic regression analysis was employed when effects of the immunostaining of p16 and p21 on the cytological diagnosis were evaluated under adjustment of other confounding factors. Follow-up data were collected for 61 cases diagnosed as NILM (52 cases) and ASC-US (9 cases) at the first visit. Follow-up data were analyzed by log-rank test and Cox proportional hazard model. All participants gave an informed consent and the study protocol was approved by the local ethics committee of the NHO Hamada Medical Center.

RESULTS AND DISCUSSION

Cases with positive expression of p16 and p21 increased with the cytological grade. The cases positive for both p16 and p21 were 4 and 18 % in the NILM and LSIL, respectively, and this incidence was as high as 70 % in HSIL. This implied that the double staining for p16 and p21 was a good marker for HSIL. In contrast to immunocytochemistry for p16, which was often positive in early stages of SIL, p21 seemed to be positive in the higher grades of SIL. These results indicated that immunocytochemistry for p16 and p21 might be useful markers in the diagnosis of SIL in the cytological examination.

It is of interest that the integrated (I) > episomal (E) pattern in ISH prevailed in HSIL cases (93 %) while the E≥I pattern was mainly seen in LSIL cases (87 %). For the discrimination between LSIL and HSIL, "p16 (+) and p21 (+)" and "I>E pattern in the ISH" were the best in the immunocytochemistry and the HPV genomic tests, respectively.

Under adjustment of the patients' age and the history of cervical intraepithelial neoplasia, the immunocytochemistry for p16 and p21 was a significant discriminator for the cytological grade both between HSIL/LSIL and NILM, and between HSIL and LSIL/NILM by multiple logistic regression analysis. Furthermore, even in combination with the genomic tests of HPV, the immunocytochemistry was still an independent factor discriminating the cytological grades.

To evaluate usefulness of p16 and p21 immunocytochemistry as well as the genomic tests

of HPV in the prediction of the disease progression, we performed a prospective study on 61 NILM/ASC-US cases. We found progression of the disease in 20 cases during the follow-up period. Log-rank test indicated that the infection to high-risk HPV as well as the cytological diagnosis (ASC-US vs. NILM) influenced the prognosis significantly (p=0.007 and 0.002, respectively). On the other hand, status of the p16 and p21 expression by immunocytochemistry did not reach a significant level. Cox proportional hazard model indicated that the infection to high-risk HPV as well as the cytological diagnosis (ASC-US) influenced the prognosis significantly (p=0.007 and 0.001, respectively). On the other hand, positive staining of p16 or p21 was not found to be an independent risk factor.

The present study showed that the positive staining for p16 and p21 was additional good marker to distinguish the higher grade SILs. An additional analysis indicated that the sensitivity and specificity of 'double positive for p16 and p21' predicting the I>E pattern in the ISH were 62% (21/34) and 90% (103/115), respectively. These results suggested that, even in the HPV-positive cases, immunostaining for p16 or p21 was useful to predict the genome integration of HPV, which might indicate a greater risk for progression to cancer.

Although the present study failed to establish the superiority of the immunostaining alone versus the HPV genomic tests in the screening, the combination of the immunostaining and HPV genomic tests may be useful in routine cytological examinations.

CONCLUSION

In conclusion, the present study indicated that immunostaining for p16/p21 in cytological specimens might provide additional supportive information in the diagnosis of SIL. The significance of the immunostaining of p16/p21 in the prediction of the prognosis of cases with HPV infection needs to be established in a future study using a larger population.