

# 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<sup>INK4a</sup> / p21<sup>WAF1/CIP1</sup> 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<sup>INK4a</sup> (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<sup>WAF1/Cip1</sup> (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.

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## 論文審査の結果の要旨

近年若年層で罹患率が急増している子宮頸癌は、無症状時から細胞診による子宮頸がん検診を受け、早期発見する事が重要である。子宮頸がん検診に用いられる細胞診分類には、最近ベセスダ分類が採用されたが、本分類の症例管理法や進展予測には不明の点が多い。サイクリン依存性キナーゼ阻害タンパク質である p16 と p21 は、子宮頸部扁平上皮内病変の進展に密接に関与している。本研究では、倫理委員会承認のもとで、患者 149 症例の横断的検討と、細胞診で異常なしか軽微な異常症例のうちで追跡調査ができた 61 症例 (213 検体) の縦断的検討を、免疫染色 (p16, p21)、human papillomavirus (HPV) genotyping、high risk HPV-in situ hybridization を用いて行った。その結果、①横断的研究では、p16, p21 免疫染色により細胞診で扁平上皮内病変を診断する際に有用な補助的情報を得られることが明らかとなった。特に p16 は初期病変、p21 はより進行病変の鑑別に有用であり、HPV 検査と組み合わせても有用性は失われなかった。②縦断的検討では、HPV 感染の有無、細胞診による形態学的診断が独立した扁平上皮内病変の進展予測因子となったが、p16、p21 免疫染色は有用な傾向はあったが統計的有意とはならなかった。以上の成績は、子宮頸がん検診において低コストで簡易な免疫染色が症例管理のための補助的情報として有用である可能性を明らかにしたもので、臨床的に有用な新知見であり学位授与に値すると判断した。