学 位 論 文 の 要 旨

氏名　飯田 幸司

学 位 論 文 名　EGFR Gene Amplification Is Related to Adverse Clinical Outcomes in Cervical Squamous Cell Carcinoma, Making the EGFR Pathway a Novel Therapeutic Target

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著 者 名　Kouji Iida, Kentaro Nakayama, Mohammed Tanjimur Rahman, Munmun Rahman, Masako Ishikawa, Atsuko Katagiri, Shamima Yeasmin, Yoshiro Otsuki, Hiroshi Kobayashi, Satoru Nakayama, Kohji Miyazaki.

論 文 内 容 の 要 旨

INTRODUCTION

Uterine cervical cancer is the second most common malignancy among women worldwide. Despite the availability of screening, cervical cancer is still a leading cause of cancer death in Japanese women. This is partly because some patients continue to present with advanced-stage disease for which conventional therapy is less effective. Therefore, novel therapeutic agents are urgently needed to improve the outcome in these patients. Human papillomavirus (HPV) is the aetiologic agent of cervical cancer; however, HPV infection is not sufficient. Alterations in oncogenes and tumor-suppressor genes in cervical cells are essential for cervical carcinogenesis. Amplification of DNA in certain chromosomal regions is one of the mechanisms by which genes that are critical in the development and progression of human cancers are activated. In squamous cervical cancers in particular, proto-oncogenes, such as *EGFR* (7q12), *MYC* (8q24), *ERBB2*(17q11.2-12), *CCND1* (11q13), *HRAS* (11q15.5), and *cIAP1* (11q22) are often activated by amplification. The gene for the epidermal growth factor receptor (EGFR) maps to 7p11.2-p12 and comprises 28 exons, which encode a protein containing an extracellular ligand-binding domain, a transmembrane domain, and a tyrosine kinase domain. Epidermal growth factor receptor was the first tyrosine kinase transmembrane receptor to be directly linked with human cancer. Previous studies have shown EGFR to be frequently overexpressed in primary cervical cancer. however, the mechanism of EGFR activation (i.e., gene amplification or activating mutation) in cervical cancer is poorly understood. Additionally, the EGFR/RAS/RAF/MEK/ERK

pathway and its downstream effectors have primarily been studied in the context of squamous cell carcinomas, which comprise 85–90% of cervical cancers. It is unclear whether EGFR amplification is a feature of adenocarcinomas and adenosquamous carcinomas, which comprise 10–25% of cervical cancers. The aim of this study was to investigate the differences in EGFR overexpression, *EGFR* gene amplification, and activating mutations in the tyrosine kinase (TK) domain of this gene between squamous cell carcinomas and adenocarcinomas/adenosquamous carcinomas of the uterine cervix. In addition, we compared the phenotypes in cultured cervical cancer cells with various EGFR expression levels after treatment with the potent EGFR inhibitor AG1478.

MATERIALS AND METHODS

A total of 59 paraffin-embedded cervical squamous cell carcinomas tissue samples were obtained from the Department of Obstetrics and Gynecology at Shimane University Hospital. Also, 52 paraffin-embedded adenocarcinomas/adenosquamous carcinomas tissue samples were obtained from the Department of Obstetrics and Gynecology at Seirei Hamamatsu General Hospital. Patients had received appropriate therapy at either Shimane University Hospital or Seirei Hamamatsu General Hospital between January 1994 and December 2007. The EGFR expression, amplification, and mutation in cervical carcinomas were assessed by immunohistochemistry, fluorescence in situ hybridization, and PCR-SSCP, respectively, and correlated with clinical data collected by a retrospective chart review. A functional assessment was performed by inactivating EGFR in cervical cancer cells with the potent inhibitor AG1478. Acquisition of tissue specimens and clinical information was approved by an institutional review board (Shimane University and Seirei Hamamatsu General Hospital).

RESULTS AND DISCUSSION

Immunohistochemical analysis revealed that 6 out of 59 (10.2%) cervical squamous cell carcinomas showed significant amplification of the *EGFR* locus, whereas none of the 52 adeno/adenosquamous cell carcinomas had detectable *EGFR* amplification ($P<0.05$). The *EGFR* amplification significantly correlated with shorter overall survival ($P=0.001$) in cervical squamous cell carcinomas. Multivariate analysis showed that *EGFR* gene amplification was an independent prognostic factor for overall survival ($P=0.011$). None of the squamous cell carcinomas (0%: 0 out of 32) had detectable oncogenic mutations in *EGFR* exons 18 through 21. The frequencies of *KRAS* and *BRAF* mutations were very low in both squamous and adeno/adenosquamous cell carcinomas. Sensitivity of cervical cancer cells to AG1478 depended on the presence of EGFR overexpression. AG1478-induced EGFR inactivation in cell lines with EGFR overexpression significantly suppressed tumor development and progression in a mouse xenograft model.

The higher frequency of EGFR expression in squamous cell carcinomas compared with adenocarcinomas/adenosquamous cell carcinomas is a finding of interest. It suggests that adenocarcinomas/adenosquamous carcinomas may be distinguished from squamous cell carcinomas based on characteristic genetic alterations. As EGFR overexpression is more prevalent than *EGFR* gene amplification, we sought to investigate whether activating mutations could constitute an alternative mechanism for EGFR overexpression as this has been reported in other solid tumors. We did not identify activating mutations in the tyrosine kinase domain of 32 cervical squamous cell carcinomas. Our results are in agreement with previous studies demonstrating the lack of *EGFR*-activating mutations in breast cancer. Furthermore, in the present study only the *EGFR* TK domain was analyzed. Although exons 18–21 are the hot spot region for *EGFR* gain-of-function mutations , activating mutations in other domains of the gene cannot be excluded. Recent reports have shown that *EGFR* mutations are rare or occur at a very low frequency in acute leukaemia, glioblastoma, and colorectal, gastric, breast, and hepatocellular carcinomas. Although 80% of lung cancer patients who are carriers of *EGFR* TK domain mutations experience partial responses or marked clinical improvement with gefitinib or erlotinib, patients without such mutations are refractory to these agents. In the present study, we demonstrated that cervical squamous cell carcinoma cell lines with *EGFR* amplification were more sensitive to a potent EGFR inhibitor AG1478, which suggests that TKI therapy may have some utility in cervical cancer tumors without mutations, provided that *EGFR* amplification is present. Cetuximab, a chimeric IgG1 monoclonal antibody, and panitumumab, a fully humanised IgG2 monoclonal antibody, belong to a new generation of drugs that block extracellular ligand binding to EGFR. Cetuximab, an FDA-approved drug, has shown promising results in colorectal and head and neck cancers. Furthermore, cetuximab has antitumor activity in NSCLC models expressing both wild-type and mutated *EGFR*. Cervical cancer cell lines derived from primary and recurrent tumors have also been shown to be very sensitive to cetuximab-mediated antibody-dependent cellular cytotoxicity and to cetuximab-mediated inhibition of tumor growth. Although studies in colorectal carcinoma have shown that somatic *KRAS* mutation is associated with resistance to cetuximab, the present study has shown *KRAS* mutations to be rare in cervical carcinomas. On the basis of these findings, cetuximab therapy may be efficacious in cervical carcinoma patients who have EGFR protein overexpression without *KRAS* mutations, particularly those who have not responded to standard treatment modalities.

CONCLUSION

This study suggest that EGFR signalling is important in a subset of cervical squamous cell carcinomas and that anti-EGFR therapy may benefit patients who carry *EGFR* gene amplification in their tumors.