学 位 論 文 の 要 旨

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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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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.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

進行性子宮頸癌は従来の治療では奏効率が低く，新規治療法の開発が強く望まれる疾患である。申請者らは，子宮頚癌における上皮細胞増殖因子受容体（EGFR）に着目し，インフォームドコンセント及び倫理委員会で承認の得られた子宮頚癌検体を用いて，その遺伝子増幅，タンパク質発現および遺伝子変異を評価し，臨床病理学的所見と予後との関連性から潜在的治療戦略としての EGFR の意義を解析した。また，子宮頸癌細胞株では *EGFR/KRAS/BRAF* 遺伝子の変化と EGFR 阻害剤 AG1478 の感受性変化を併せて評価した。FISH 法では子宮頸部扁平上皮癌の10.2%に *EGFR* 遺伝子の増幅を認め，*EGFR* 遺伝子の増幅を呈する扁平上皮癌症例では全生存率が有意に低下していた(p=0.001)。多変量解析でも同様で，EGFR 遺伝子の増幅は子宮頸部扁平上皮癌の独立した予後規定因子であった(p=0.011)。一方，EGFR の下流に存在する *KRAS* あるいは *BRAF* 遺伝子の突然変異を一本鎖高次構造多型分析法あるいは DNA シークエンス法で評価したが，いずれも子宮頸部扁平上皮癌では観察されなかった。*EGFR* 遺伝子が増幅を呈する子宮頚癌細胞株（SKGIIIa，CaSki，ME180）では，遺伝子増幅のない細胞株に比較し EGFR 阻害剤AG1478 に対する感受性が高く，ヌードマウスを用いた異種移植モデルでも同様の結果が得られた。以上より，子宮頸部扁平上皮癌では *EGFR* 遺伝子の増幅が予後不良の指標となり，新しい戦略として EGFR を標的とする治療法の可能性が示唆された。

最終試験又は学力の確認の結果の要旨

申請者は，進行性子宮頸部扁平上皮癌における *EGFR* 遺伝子増幅と生物学的悪性度の解析結果から，EGFR 標的治療が子宮頸部扁平上皮癌の新しい治療戦略となる可能性を明らかにした。発表も適切であり，質疑応答も的確であったので，学位授与に値すると判断した。

（主査：椎名浩昭）

申請者は，子宮頸部扁平上皮癌における *EGFR* 遺伝子増幅を FISH 法を用いて評価し，さらに細胞株における EGFR 阻害薬の効果から EGFR が分子標的となり得ることを示した。公開審査における質疑応答も的確で，関連分野の知識も豊富であり，学位授与に値する。

（副査：浦野　健）

申請者は，子宮頸部扁平上皮癌の発癌機構で *EGFR* 遺伝子増幅は独立予後因子で予後不良であることを示した。公開審査における質疑応答も的確で，関連分野の知識も豊富であり，学位授与に値すると判断した。

（副査：大平明弘）

（備考）　要旨は、それぞれ４００字程度とする。