

## 論文要旨等報告書

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### 学位論文内容の要旨

**Background:** Nowadays head and neck squamous cell carcinoma (HNSCC) is the sixth most frequent cancer world wide. Over activation of the EGFR-RAS-RAF-MAPK signaling cascade has been implicated in the development of various cancers including HNSCC. Recently molecular targeted therapy has gained a lot of interest in the treatment of cancer. Since mutation analysis of the signaling molecules in this pathway may have clinical implications in targeted therapy, we have analyzed the mutation status of three main molecules related to this pathway, namely EGFR, ErbB2 and K-ras. Expression of EGFR was also analyzed using immunohistochemistry analysis.

**Objectives:** In lung cancer it has been reported that mutations in the tyrosine kinase domain of EGFR are associated with a better response to targeting therapy using tyrosine kinase inhibitors. Since a response to these agents could also be detected in HNSCC, the objective of this study was to analyze the mutation status of the EGFR and other related signaling molecules in HNSCC patients. This might have useful clinical applications in evaluating a possible response of these patients to targeting therapy and may aid in screening of the HNSCC patients. Overexpression of EGFR could also lead to similar effects as activating mutations and previous reports have yielded controversial results in this regard, thus we also analyzed the expression of EGFR in these patients to evaluate its expression status as well as its usefulness as a prognostic marker in these patients.

**Materials and Methods:** 91 Japanese HNSCC patients and 12 HNSCC cell lines (provided by Dr. Grenman R) were analyzed for mutations in EGFR, ErbB2 and K-ras. The exons encoding the hot spot regions in the TK domain of both EGFR (exon 18, 19, 21), ErbB2 (exons 18-23), as well as exon 1 and 2 of K-ras gene were amplified by PCR and directly sequenced for mutation analysis. Expression of EGFR was also analyzed in 65 HNSCC patients using Immunohistochemistry. Statistical analysis was performed to analyze the relation between EGFR expression and the patient's clinical variables. Survival analysis was also performed using the Kaplan-Meier method.

**Results:** Only one silent mutation C836T was found in exon 21 of EGFR in UT-SCC-16A cell line and its corresponding metastasis cell line UT-SCC-16B. No other mutation was found in EGFR, ErbB2 or K-ras gene. All tumors have shown expression of EGFR. In 21 (32%) tumors EGFR was weakly expressed (+1). In 27 (42%) tumors it was moderately expressed (+2) and in 17 (26%) tumors high expression (+3) was detected. Overexpression (+2, +3) was found in 44 tumors (68%). A worse tumor differentiation and a positive nodal stage were significantly associated with over expression of EGFR ( $p=0.02$  and  $p=0.032$  respectively).

**Conclusion:** EGFR might be considered as a marker of poor prognosis in HNSCC. Activating mutations of EGFR, ErbB2 and K-ras are absent or rare in Japanese HNSCC patients. Protein over expression rather than mutation might be responsible for activation of the EGFR pathway in HNSCC patients.

## 論文審査結果の要旨

頭頸部扁平上皮癌は世界中で6番目に多い癌である。近年、その分子標的治療のターゲット分子として、epidermal growth factor receptor (EGFR)が注目されている。EGFRはErbB family受容体の一つである膜貫通型蛋白であり、RAS-RAFを介したMAPKカスケードは癌の進展に重要な役割を果たすことが知られている。肺癌においてはEGFR遺伝子のtyrosine kinase domainにおけるmutationが報告されており、tyrosine kinase阻害剤であるgefitinibの抗腫瘍効果との関連が示唆されている。

本研究は、頭頸部扁平上皮癌におけるEGFR、ErbB2および下流シグナル分子であるK-ras、B-rafのmutation解析を行ったものである。さらに、EGFR蛋白の発現と臨床データとの関連について検討している。

頭頸部扁平上皮癌91症例および扁平上皮癌由来細胞株12株を用い、mutation解析を行った結果、2細胞株においてEGFRのsilent mutation (C836T)が確認された。しかし、ErbB2、K-ras、B-rafのmutationは認められなかった。

免疫組織化学的解析によりEGFR蛋白の発現を検索した結果、66症例中44症例(68%)において過剰発現を認めた。臨床データとの比較から、EGFRの過剰発現は癌の分化度およびリンパ節転移と相関することが示された。

以上のことから、頭頸部扁平上皮癌では、EGFR pathwayのmutationはほとんどみとめられず、蛋白の過剰発現がEGFRシグナルの活性化と関連していることが示唆された。また、蛋白の過剰発現は癌の病態と相関することが示唆された。

これらの知見は、頭頸部扁平上皮癌における病態解明の一端を担う基礎研究として価値のある研究業績である。よって本論文は、博士(歯学)の学位授与に値すると判断した。