学 位 論 文 の 要 旨

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学 位 論 文 名　Association of EPAS1 Gene rs4953354 Polymorphism With Susceptibility to Lung Adenocarcinoma in Female Japanese Non-Smokers

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論 文 内 容 の 要 旨

INTRODUCTION

Evaluation of the genetic factors underlying the development of lung cancer can elucidate the etiology of the disease and identify high-risk individuals for targeted screening and/or prevention.

The transcription factor hypoxia-inducible factor-α (HIF- α) plays an important role in the regulation of hypoxic signaling. It has also been reported to be involved in the progression of solid tumors and associated with various aspects of carcinogenesis, including tumor metabolism, angiogenesis and metastasis. Although HIF-1 α and HIF-2 α (also called endothelial PAS domain protein 1: EPAS1) have similar functions in vitro, several recent in vivo studies on the pathophysiological roles of HIF-2 α have described its role in angiogenesis during human fetal lung development, especially in the last phases of pregnancy, preparing the fetus for extrauterine life. In addition, high expression of HIF-2 α is reported to be associated with poor outcome in various cancers, including lung cancer. However, whether HIF-2 α is involved in the lung carcinogenesis remains elusive at present.

In the present study, we focused on the EPAS1 (HIF-2 α) gene rs4953354 polymorphism that has been reported to contribute to adaptation to high-altitude hypoxia in

Sherpas. We conducted a case-control study to explore a possible association of the rs4953354 polymorphism with susceptibility to non-small cell lung cancer (NSCLC) in Japanese population, with a special emphasis on adenocarcinoma developed among female never-smokers.

MATERIALS AND METHODS

A case-control study of 346 patients with NSCLC (adenocarcinoma=249, squamous cell carcinoma=97) and 247 healthy control subjects was carried out. Genomic DNA was isolated from peripheral total blood cells or surgically resected normal tissues adjacent to cancers. Each participant provided written informed consent for the collection of blood and/or tissue samples and subsequent analysis. Ethical approval was obtained from the Institutional Review Board of Shimane University Faculty of Medicine, Higashihiroshima Medical Center, and Hiroshima University Faculty of Medicine.

PCR was performed to amplify the human *EPAS1* gene using specific primer set and conditions. The following primer set was used to amplify a 281-bp fragment of the EPAS1, which contains polymorphic allele A/G (rs4953354): forward 5' - CTG GGA AAG AGG GAA TCC AGT GTG - 3' and reverse 5' - CTC AGC CCA CTG TTC TCT CTT TGC - 3'. The PCR reaction conditions were 40 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s and extension at 68°C for 30 s. Direct sequence analysis was carried out using these primers with Big Dye Terminator Cycle Sequencing Kit™ and ABI PRISM 310 Genetic Analyzer™ (Applied Biosystems, Foster City, CA).All the statistical analyses were conducted, unless otherwise specified, using JMP 9.0.3 (SAS Institute, Cary, NC).

We used statistical language and environment, R for calculation of extended Fisher's exact test for 2x3 tables. *P* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The control subjects were slightly younger than the patients, while no difference was observed for gender distribution and smoking status. The distribution of A/G alleles at rs4953354 among the control subjects fulfilled the Hardy-Weinberg equilibrium.

Lung adenocarcinoma was significantly more frequent in patients with *G* allele at the rs4953354 (odds ratio [OR]=1.607; 95% confidence interval [95%CI], 1.039-2.484; *P*=0.032), compared with normal controls with other genotypes, especially in female never-smokers (OR=3.31; 95%CI, 1.21-9.01; *P*=0.017). Mutations in epidermal growth factor receptor

(EGFR) tended to be frequent in patients with *G* allele at the rs4953354, compared to those with other genotypes.

To the best of our knowledge, this is the first study that identified a useful biomarker for the risk of lung adenocarcinoma in female never-smokers. The results showed that the *G* allele at the rs4953354 locus of *EPAS1* polymorphism is associated with significantly high OR for lung adenocarcinoma in female never-smokers, compared to normal controls with other genotypes.

Although the mechanisms underlying the high risk was not determined, EPAS1 (HIF-2 α) may contribute to lung carcinogenesis *via* the HIF signaling pathway, such as anti-apoptosis, tumor angiogenesis, modified energy metabolism and genomic instability.

Interestingly, our results showed that the frequency of adenocarcinoma patients with at least one *G* allele tended to be higher in those with *EGFR* mutations. Although there is no report examining the relationship between *EGFR* mutations and HIF-2α, a positive regulatory loop between EGFR and HIF signals may exist. Database analysis showed that the rs4953354 is located close to the binding sites of several transcription factors, as identified by the ChIP sequence, suggesting that the SNP might affect *EPAS1* expression. Altered *EPAS1* activity by the *G* allele at the rs4953354 might be involved in not only activated HIF signals but also induced *EGFR* mutations, which could increase the susceptibility to lung adenocarcinoma. Thus, our study identified a potentially useful biomarker for high-risk individuals, i.e., female never-smokers, for lung adenocarcinoma. The results also enhance our understanding of the molecular mechanisms of lung carcinogenesis in such patients.

Our study has some limitations. Because the majority of the control subjects were recruited from annual health examinations, age of control did not match that of the patients, although it was adjusted to be close each other. Smoking history of control was also lacked. Further, the number of female never-smokers with lung adenocarcinoma was relatively small. To verify the relationship between the SNP and *EGFR* mutation, a larger number of female never-smokers with lung adenocarcinoma is needed.

CONCLUSION

The *EPAS1* rs4953354 is a potentially useful genetic marker for lung adenocarcinoma in high-risk groups, especially female never-smokers.

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］

原発性肺癌の治療は、特に、非小細胞肺癌では、劇的に進歩している。それは、Epidermal growth factor receptor(EGFR)遺伝子などの発癌ドライバー遺伝子変異に対する分子標的治療が可能になったためである。そこで、原発性肺癌の分子マーカーの探索が行われている。

がん組織は低酸素環境にあり、低酸素で誘導される転写因子hypoxia-inducible factor（HIF）は、様々な癌関連シグナルと相互作用することによって、発癌、悪性度増大などにかかわる。しかし、HIFと肺癌患者の疾患感受性との関連は明確にされていない。本研究では、HIF-2α（EPAS1）のSNP（rs4953354）と肺癌疾患感受性の症例対照研究を行った。

対象は非小細胞肺癌患者346例（肺腺癌247例，肺扁平上皮癌97例）と健常対照247例である。対象者の末梢血または正常組織からDNAを抽出し、PCR法を用いてHIF-2α（EPAS1）遺伝子の増幅を行い、直接塩基配列決定法を用いて遺伝子多型の検討を行った。

rs4953354 のGアレルと肺癌の頻度の関連については、肺腺癌の女性の非喫煙者群において、有意にGアレルを有する患者が多かった。EPAS1のrs4953354のGアレルが、肺腺癌の疾患感受性を増大させる可能性が示唆された。

EPAS1のSNPは、非喫煙女性における肺腺癌の潜在的な危険因子として、CT検診など感度の高い肺癌スクリーニングと組み合わせることで、高リスク群を選定できる可能性がある。

［最終試験又は学力の確認の結果の要旨］

申請者は、内皮細胞、腎、肺などで発現し、脱分化、細胞浸潤、細胞周期などに関わる転写因子であるHIF-2αのSNP（rs4953354）が非喫煙者の女性における肺腺癌の潜在的な危険因子であることを明らかにした。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。　（主査：吉山　裕規）

申請者は低酸素応答の制御遺伝子である*HIF-2α*遺伝子の一塩基多型が非喫煙女性の肺腺癌の疾患感受性を増大させることを症例対照研究により証明した。また、*HIF-2α*遺伝子の一塩基多型による新たな肺がんの診断戦略の可能性も示した。関連知識も豊富で、質疑にも的確に答え、学位授与に値すると判断した。　（副査：椎名　浩昭）

申請者は、低酸素応答機構に関与するHIF-2α(EPAS1)のSNP（rs4953354）に関して、肺癌疾患感受性との関連について検討し、肺腺癌の高リスク群のスクリーニングに応用可能な重要な知見を示した。また関連知識も豊富で質疑応答も的確なため、学位授与に値するものと判断した。（副査：松本　健一）