論 文 要 旨 等 報 告 書

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学位論文題名	C-kit Protein Expression Correlated with Activating Mutations In KIT Gene in Oral Mucosal Melanoma (口腔悪性黒色腫におけるKIT遺伝子機能獲得型突然変異とc-kit蛋白発現に関する研究)
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学 位 論 文 内 容 の 要 旨

Background: Oral mucosal melanoma (OMM) is an extremely rare neoplasm representing about 0.5% of oral malignancies and less than 0.01% of all oral biopsies. OMM is a formidable and fatal neoplasm expressing a plethora of molecules indispensable for steadfast invasion, growth, proliferation and metastasis. Among these molecules were heparanase, which had been implicated in tumor invasion and vascular endothelial growth factor (VEGF) and its receptor (VEGFR-2), which were immensely associated with angiogenesis. Although, the interactions of these molecules may regulate the aggressive behavior of OMM, a clearer understanding of the mechanism behind its aggressiveness could lead to therapies that may block the carcinogenic process especially during the early stage and keep local disease in control. C-kit is a member of the receptor tyrosine kinase (RTK) family that influences proliferation, migration and survival of melanocytes. It is a protein encoded by the proto-oncogene KIT and is activated by its ligand, stem cell factor (SCF) during melanogenesis. Gain-of-function mutations arising to c-kit activation independent of its ligand were observed in various tumors especially in gastro-intestinal stromal tumors. The activating mutation induced tremendous proliferation, inhibited apoptosis and instigated oncogenesis. C-kit may also induce VEGF expression leading to angiogenesis.

Objectives: Since c-kit is an important receptor for melanocyte growth and maturation, its function might be inferred in OMM. Moreover, the detection of c-kit activating mutation may give an insight to the value of specific RTK inhibitor in a subset of melanoma. Thus, the c-kit protein expression and activating mutations in exons 11 and 13 were investigated. The correlation between increase protein expression and the presence of activating mutation was also analyzed.

Materials and methods: Eighteen cases of OMM were used for the study. Immunohistochemistry was performed using c-kit antibody and the expression was determined as negative (-), focal (±), moderate (+) or intense (++). Mutation analysis was performed by

extracting genomic DNA from paraffin blocks and amplifying exons 11 and 13. P CR products were subjected to single strand confirmation polymorphism (SSCP) and the by direct sequencing.

Results: C-kit expression was detected in atypical melanocytes. Furthermore, c-kit expression was observed in all in situ components ranging from moderate to intense (only 1 case had focal expression). In invasive component, c-kit expression was observed in 16/18 cases but only four cases had intense expression. SSCP gels for exons 11 and 13 revealed many aberrant bands. When these bands were sequenced, missense and silent mutations were observed. Missense mutations were observed in 4/15 cases (27%) with DNA amplification. Two mutations were observed in cases with increased protein expression. One mutation was observed in a case with several recurrences and another was observed in amelanotic case.

Discussion and conclusion: OMM has a poor prognosis with a 5-20% survival rate. Analysis of its molecular biology is one important leap towards understanding the nature of the tumor and for the provision of an ample management. Atypical melanocytic proliferation may indicate an early progression to OMM. Therefore, the expression of c-kit in atypical melanocytes suggests the role of c-kit in the early stage of OMM tumorigenesis. Although a decrease in c-kit protein from in situ to invasive phase was observed, an increased expression was detected in two cases, which incidentally conformed to the presence of missense mutations suggesting the correlation between increased c-kit protein and presence of activating mutation. Moreover, one mutation was found in a case with several recurrences and another was observed in an amelanotic case. These results suggest that c-kit has a function in the aggressiveness of OMM. In addition, VEGF expression might be regulated by c-kit signal transduction cascade. Several RTK inhibitors have anti-tumor effects by blocking c-kit activation as well as suppressing VEGF transcription and translation. Since VEGF is highly expressed by OMM cells, the use of specific RTK may block c-kit activation as well as suppress angiogenesis. In summary, c-kit expression suggests a role in the early stage of OMM tumorigenesis. C-kit protein expression correlated with activating mutations suggesting the pertinent role of the proto-oncogene KIT in the tumorigenesis of OMM.

論文審査結果の要旨

C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma

口腔悪性黒色腫は高い侵襲性、転移能を示すメラノサイト由来の悪性腫瘍であり、その病態解明が強く望まれる。口腔悪性黒色腫の発育は上皮内の水平的増殖から上皮下への垂直的増殖を示し、異型メラノサイト増殖、上皮内悪性黒色腫、浸潤性悪性黒色腫へと進行する。

C-kit蛋白は、染色体4q11-12に存在するKIT遺伝子によってコードされる膜貫通型受容体チロシンキナーゼであり、リガンドであるstem cell factorと結合することで細胞内へシグナルを伝達する。GIST（胃腸管間質腫瘍）や皮膚の悪性黒色腫においてc-kit蛋白の発現やc-kit遺伝子の突然変異が報告され悪性黒色腫への関与が示唆されているが、口腔悪性黒色腫に関しては不明である。

本研究の目的は、口腔悪性黒色腫におけるc-kitの関与を明らかにすることであり、免疫組織化学的手法およびmutation解析を用いて検討したものである。

口腔悪性黒色腫18症例を用いたc-kit蛋白の免疫組織化学的検索により、16症例で浸潤性悪性黒色腫の腫瘍細胞に蛋白の局在を認めた。異型メラノサイト増殖や上皮内悪性黒色腫を示す部位においても陽性であり、c-kit蛋白が初期の段階から悪性黒色腫に関与することが示された。

C-kit遺伝子のmutation解析の結果、4症例において1種類の新規を含む5種類のmutationを認めた。4症例のうち1例は複数回再発を起こした症例、1例は悪性度が高いとされる無色素性悪性黒色腫であり、mutationと悪性度との相関が示唆された。また、2例では免疫組織化学的結果において強い染色性を示し、蛋白発現との関連が示唆された。

以上のことから、口腔悪性黒色腫においてc-kit蛋白が腫瘍の初期段階から重要な役割を果たし、c-kit遺伝子の突然変異が蛋白発現や腫瘍の生物学的性状に深く関与することが示唆された。

これらの知見は、口腔悪性黒色腫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（学術）の学位授与に値すると判定した。