

## 論文要旨等報告書

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学位論文題名	Fine deletion mapping of chromosome 2q21-37 shows three preferentially deleted regions in oral cancer (口腔癌における染色体2q21-37部位の微調欠失マッピング)

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### 学位論文内容の要旨

#### Introduction;

Cancer is basically a genetic disease. The collection of genetic and epigenetic alterations of multiple genes and chromosomes lead to the development of cancer. Among these changes, inactivation of the tumor suppressor gene (TSG)s is one of the most critical steps. The deletion of targeted chromosomal region eliminates one allele, while inactivating events (mutation, deletion or promoter hypermethylation) affect the other allele of the concerning TSG. Loss of heterozygosity (LOH) analysis is a sensitive method for monitoring deleted region on chromosomes. The LOH assay is designed to assess polymorphic chromosomal regions that map close to or within putative or known TSGs. We recently prepared genome-wide deletion mapping of all chromosomes by using 191 microsatellite markers in head and neck cancer. This initial screening study revealed highly deleted regions on chromosome 2 such as 2q22.1, 2q23.1, 2q33.1 and 2q37.3.

Since the human genome sequence project has been completed, we now have more precise and detailed information about the location of genes and microsatellite markers. Thus, we examined a commonly deleted region of chromosome 2q21-37.3 in detail by using 16 microsatellite markers and constructed a deletion map of the region and tumor suppressor gene (TSG)s.

**Materials and Method;** Paired normal and tumor samples were obtained from 39 patients with the diagnosis of primary oral squamous cell carcinoma, at Okayama University Hospital after acquisition of informed consent from each patient. Genomic DNAs were isolated from frozen tissues by SDS/proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation. We used 16 polymorphic microsatellite markers for PCR amplifications. Primers for amplification of microsatellite markers D2S1328, D2S2304, D2S111, D2S115, D2S116, D2S202, D2S155, D2S1327, D2S164, D2S133, D2S427, D2S206, D2S336, D2S338, D2S125 and D2S140 are available through the internet genome database. (<http://gdbwww.gdb.org/>). After amplification, 2-4  $\mu$ l of the reaction mixture were mixed with 8  $\mu$ l of loading dye heat denatured, chilled on ice, and then electrophoresed through an 8% polyacrylamide gel containing 8 M urea. The DNA bands were visualized by silver staining as described previously.<sup>3,5</sup> LOH was scored if one of the heterozygous alleles showed at least 50% reduced intensity in tumor DNA as compared with the corresponding normal DNA as described previously.

**Result;** Overall, LOH was detected at least in one location in 33 of 39 (85%) tumor tissues on chromosome 2q21-37.3 region. The result of LOH analysis revealed frequent allelic loss of at least 3 separate locations in the region (Figure 1A). Three cases (7-25, 9-10 and 20-18) showed a large deletion that included most polymorphic markers tested. In the other 30 cases, a partial deletion was found. The high frequency of LOHs was observed at loci on D2S2304 at 2q21-24(%35), D2S111 at 2q24(%40), D2S155 at 2q33 (%35), D2S1327 at 2q32-35 (29%), D2S164 at 2q35 (29%), D2S125 at 2q37.3 (%68) and D2S140 at 2q37.3 (%32). Three different regions preferentially have been lost. Five cases showed loss only at D2S125, the highest marker of LOH,

with the retention of flanking markers. In the second hot spot, the markers D2S155, D2S1327 and D2S164 were deleted with retention of the flanking markers narrowing down the targeted area. The region spanned 120 Mbp distance between the markers D2S1328 and the most telomeric side of the long arm. Three selective areas were lost within this large chromosomal region, which included several candidate TSGs such as LRP1B, CASP10, CASP8, ADAM23, BARD1, ILKAP, PPP1R7, STK25, BOK, and ING5.

**Discussion;** The functional loss of tumor suppressor genes is closely related with the initiation and/or progression of human cancer. Recently, there are increasing numbers of reports presenting deletion on chromosome 2q in various cancers. Our data revealed high frequency of LOH in three different regions on chromosome 2q21-24 (%40), 2q33-35 (%35) and 2q37.3 (%68).

This is the most comprehensive report that shows allelic loss of heterozygosity on chromosome 2 in oral cancer. The highest LOH was detected at the marker D2S125. We previously detected the location of D2S125 at 2q37.3 region as a novel hot spot in head and neck cancer. Only few papers included the 2q37 region in the LOH analysis. In one study, Narayan et al. demonstrated two minimally deleted regions, 2q35-q36.1 and 2q36.3-q37.1, in cervical carcinoma by comparative genomic hybridization. Their study also demonstrated frequent LOH at the marker D2S125 with a 42% loss. However, we used one more telomeric marker (D2S140) to the D2S125 for clarifying the extent of loss. The location of this most telomeric marker was also frequently deleted though its LOH ratio was lower than D2S125. Since we don't have any polymorphic marker beyond D2S140, any gene around D2S125 including the most telomeric area may be responsible for this loss. Several candidate TSGs such as ILKAP, HDAC4, PPP1R7, DTYMK, ING5, STK25, BOK are localized here and either each of these genes could be responsible for carcinogenesis of various cancer or a common gene within this group may be involved in their carcinogenic process. The putative TSG in this region was most likely to be around the marker of D2S125 because 5 samples showed only LOH of this marker with retention of the flanking genomic regions. PPP1R7 (OMIM# 602877) is a protein phosphatase and its genetic alterations in human cancer cell lines have been reported previously. STK25 (OMIM# 602255) is a serine/protein tyrosine kinase and is known to associate with Albright hereditary osteodystrophy and so far no study has analyzed it in a human cancer. DTYMK (OMIM# 188345) is a kinase which functions in the DNA synthesis and cell cycle. BOK (OMIM# 605404) was cloned as a pro-apoptotic Bcl-2 protein with restricted expression in reproductive tissues. ING5 (OMIM# 608525), is a member of tumor suppressor ING family (ING1-5). We previously identified two members of the family, ING1, ING3 as TSG in head and neck cancer and, we and others recently showed that a novel member, ING4, also functions as a candidate TSG. On the other hand our data showed a high frequency of allelic loss on chromosome 2q32-35. Moreover, our previous genome-wide LOH analysis also detected a frequent deletion at 2q33 region. This distance between chromosome 2q32-3 region is about 30 Mbp and consists of several candidate TSGs such as CASP8, CASP10, ADAM23, BARD1, SMARCAL1, and XRCC5. CASP10 and CASP8 have been shown to function as tumor suppressor in various cancers. Our third frequently deleted region corresponds to chromosome 2q22-23. One candidate gene could be LRP1B, which has already been proposed to function as a tumor suppressor in esophageal and uroepithelial cancers. In conclusion, we prepared deletional mapping of the long arm of chromosome 2 in detail.

Our results revealed three preferentially deleted regions and candidate TSGs on chromosome 2 in oral cancer. Future functional studies should be done to clarify the role of these candidate TSGs in oral carcinogenesis.

## 論文審査の結果の要旨

癌は癌遺伝子・癌抑制遺伝子の機能異常により発生する。特に癌抑制遺伝子の機能低下が癌の発生には重要であると考えられており、未知の癌抑制遺伝子の発見は癌化のメカニズムを解明する重要な糸口となる。

遺伝子は相同染色体にコードされているが、癌患者では癌抑制遺伝子に異常がみられ、しばしば一方の遺伝子の欠失が生じる。この遺伝子の欠失状態はLoss of Heterozygosity (以下LOH) と呼ばれ、LOHがみられる領域において癌抑制遺伝子が存在する可能性が非常に高いと考えられる。

これまでの口腔癌におけるLOHの解析から、2番染色体長腕部 (以下2q) において高頻度にLOHが存在することが明らかになっているが、その詳細は不明である。

本研究では、口腔癌において2q21-37に範囲を限定して詳細なLOH解析を行い、癌抑制遺伝子候補の検索を行うとともに、候補遺伝子と癌との関連についてmRNA発現解析、Mutation解析を用いて検討している。

口腔癌39例において2q21-37の範囲のLOH検索を行った結果、ING5の存在領域で高頻度 (68%) のLOHが認められた。INGファミリー遺伝子は癌抑制遺伝子として報告されており、p53と関連して細胞のApoptosisや癌化に関与すると考えられている遺伝子ファミリーである。従って、ING5も癌抑制遺伝子として働く可能性が考えられ、候補遺伝子としてさらに詳細な検討を行った。

ING5と癌化との関連を検討するために、ING5のmRNA発現解析、およびMutation解析を行った。その結果、Quantitative real time RT-PCRを用いたmRNAの発現量の検索では、59%の症例で発現量の低下がみられING5の機能低下が示唆された。Mutationは1症例においてpoint mutationが認められた。また、ING5には5種類のsplicing variantが存在することを初めて明らかにした。

以上の結果より、ING5遺伝子は口腔癌の癌化と密接に関連し、新規癌抑制遺伝子候補としての高い可能性が示唆された。

これらの知見は、口腔癌における癌化のメカニズム解明の一端を担う、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。