Review

Loss of heterozygosity in oral cancer

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1. Introduction

The development and progression of oral squamous cell carcinoma (OSCC) is a multistep process, which involves many genetic factors. Among them, loss of heterozygosity (LOH) studies have been used to identify regions on chromosomes that may contain putative tumor suppressor genes (TSGs). Here, we searched PubMed for relevant publications including our previous studies [1–17] and compared results of LOH in OSCCs from the articles. LOHs in OSCCs were observed at various loci on almost all chromosomes, except X and Y. In this review, the LOH in patients with OSCC and the interrelationship between TSGs and OSCC initiation and progression are discussed.

OSCC is a frequently occurring neoplasm that is usually aggressive and has a poor prognosis. Improvements in specificity and sensitivity of diagnosis and disease prognosis depend on the elucidation of the biologic and molecular mechanisms underlying carcinogenesis [18]. The accumulation of genetic alterations during oral carcinogenesis is currently explored [19–21]. However, little is known about the molecular mechanisms of OSCC compared with other human malignancies.

It has been generally accepted that loss of function of the TSG is a key event during the progression of human malignancies [22]. These genes are thought to encode proteins that negatively regulate cell growth and thus suppress tumorigenesis. Investigators have

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identified genetic alterations associated with OSCCs, such as mutations in the p53 gene [23], adenomatous polyposis coli (APC) gene [11,24], and mutation or hypermethylation of p16 [25,26]. Therefore, alternations in TSGs are widely accepted to be critical events in the multistep process leading to the development of cancer.

As allelic loss at a certain region of chromosome is thought to indicate the presence of TSGs, LOH analysis is presently the most common method used to identify potential locations for these genes [27]. Studies based on somatic LOH have already led to the discovery of several TSGs, such as the retinoblastoma (Rb) gene, APC gene [28], and fragile histidine triad (FHIT) gene [29].

The allelotyping studies, including our previous reports, have shown multiple chromosomal regions in which LOH were frequently observed in OSCCs (Fig. 1 and Table 1). This evidence indicates that there are a number of TSGs involved in the carcinogenesis of OSCC. In the present review, we also adopted a computational tool, Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Mountain View, CA, USA) to identify regulatory networks of the genes located on loci of LOH in OSCCs.

The aim of this study was to review the current knowledge of LOH in OSCC and to provide the reader with an assessment of their prognosis and predictive value.

2. Oral squamous cell carcinoma

OSCC, accounting for 275,000 new cases and more than 120,000 deaths annually, is the sixth most common malignancy and constitutes a major health problem which is associated with severe morbidity [30–32]. Despite therapeutic and diagnostic advances, the 5-year survival rate for OSCC remains at about 50% [33–35]. The poor prognosis of OSCC has not improved significantly over the past four decades [31,36]. One of the major causes accounting for the poor outcome of patients with OSCC is that a great proportion of oral cancers are diagnosed at advanced stages and, therefore, treated late. Early detection of premalignant or OSCC lesions will greatly reduce morbidity associated with late disease treatment and improve overall patient survival. Indeed, early diagnosis and treatment of OSCC lead to a mean survival rate of over 80% and a good quality of life after treatment [37].

The most important risk factors for the development of OSCC are tobacco and alcohol [38–41]. However, some patients develop OSCC without risk factors, suggesting that host susceptibility plays an important role. Therefore, dysregulation of oncogenes and TSGs, cytogenetic changes, epigenetic changes, and mitochondrial mutations associated with the development of OSCC could be important clues to preventing this disease [38,41,42].

The development of OSCCs is a multistep process accompanied by genetic and epigenetic changes, including LOH, gene inactivation by methylation, and gene amplification, all of which can alter gene expression [43]. Elucidation of the genetic changes leading to the development of OSCCs will probably result in improved molecular assays for the early diagnosis of, therapy for, and improved prognosis of this cancer. Thus, understanding the molecular mechanisms involved in the initiation and progression to malignancy of OSCC will help to improve its prognosis and in the development of new forms of treatment.

3. Tumor suppressor genes and loss of heterozygosity

Oncogenes are variations of normal genes (proto-oncogenes) that play important roles in normal cells by activating cell signaling or proliferation. These genes become active because of specific abnormalities such as gene amplification, overexpression, point mutation, insertion mutation, or translocation [44]. Another group of genes involved in cancer development and progression are TSGs [44]. TSGs have a normal physiological role of retarding cell division. TSGs work with the DNA repair system, which makes them necessary in the maintenance of host genetic stability. TSGs in mutated form can be passed on as germline heritable DNA defects. They are the cause of syndromes of genetic predisposition to cancer [45]. For example, APC, one of the TSGs, was identified 10 years ago through its association with an inherited syndrome of colorectal cancer known as familial adenomatous polyposis coli (FAP), and was mutated in the germ-line DNA of patients with FAP. Somatic mutations or allelic deletions of APC, or both, have also been described in sporadic colorectal cancer [46].

Inactivation of TSGs causes cells to display one or more phenotypes of neoplastic growth. Knudson’s definition [47] of a classical
TSG requires the inactivation of both alleles of a candidate gene in tumors. Inactivation of these classical TSGs usually occurs either in the deletion of the one own allele or mutation in the other allele. However, a class of TSGs with haploid insufficiency, in which one allele is lost and the remaining allele is haploid sufficient, has been described and these hemizygous TSGs show a tumor-prone phenotype when challenged with carcinogens [46–49].

One of the critical steps for the identification of TSGs is LOH analysis [47]. LOH is believed to be one of the key steps to carcinogenesis of several types of cancer. LOH, caused by a deletion mutation or loss of a chromosome from a chromosome pair, and identified by comparing patterns of polymorphisms in normal and tumor cells from one individual [50], is observed at many loci in cancers. At loci showing LOH, two alleles are observed in normal cells, while only one allele is detected in tumor cells because the other has been lost. When this occurs at a TSG locus where one of the alleles is already abnormal, it can result in neoplastic transformation [50–54].

LOH can be identified in cancers by noting the presence of heterozygosity at a genetic locus in an organism’s germline DNA and the absence of heterozygosity at that locus in the cancer cells. This is often done using polymorphic markers, such as microsatellites or single nucleotide polymorphisms, for which the two parents contributed different alleles [50].
Table 2
Genetic components in ingenuity networks.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Functions</th>
<th>Score*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEN, APOC3, BAP1, BLZF1, BTG3, CCND2, Cyclin A, DBP, DKK1, Estrogen Receptor, FKBPF1B, Histone h3, HKAS, IGF2R, INPB4A, KCNJ4, LGA5S, mirt-26a/mirt-26b, mirt-27b/mirt-27a, MST1, P2RX4, PLK2, PPARG2, PTER, RAFI, RB1, RNASEH1, S100A2, SHH5, SRN2, THRB, TP53, TP73, VRK1, ZNF175</td>
<td>(1) Cancer</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR1, ALDH3A1, APC, BCA1, CCNE2, CDKN1A, CDKN1C, CDKN2C, COL18A1, CYBSR, Cyclin D, DCC, DMBT1, DNM1, E2F1, FHit, HIC1, ID3, KL6, LIMA1, MAP2K3, mir-17, NFAT, PFKFB3, PHKA2, PRK2, RRM1, SHC3, SRPK2, SRC, STAT1, TOB1, TRIP6, ZVX</td>
<td>(2) Cell cycle</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>APP, NEFL</td>
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<td>4</td>
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<tr>
<td>INT52, POLR2C</td>
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<tr>
<td>5</td>
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<tr>
<td>IFNA1, IFBF, MX1</td>
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</tbody>
</table>

Bold indicates our previous reports.

* A score > 3 was considered significant.

4. Oral squamous cell carcinoma and loss of heterozygosity

4.1. LOH loci in OSCC

LOH in OSCC was first reported by Howell et al. [55]. It was then explored by many investigators. Attempts to establish the relationship between OSCCs and allele losses at different chromosome loci have been made. As shown in Fig. 1 and Table 1, we summarized previous articles regarding LOH in OSCCs. Multiple LOHs were observed at various loci on almost all chromosomes, except X and Y.

Among those chromosomes, highly frequent LOHs were observed on chromosome 3 in OSCCs [56–62]. Especially, the short arm of chromosome 3 (3p) is often deleted in OSCCs [59]. LOH has been mapped to three distinct regions of loss, 3p13–p21.1, 3p21.3–p23, and 3p25 [56–62]. The region close to 3p14 is a fragile site on 3p. At least two or even three TSGs may be involved on 3p [59]. The FHit gene, localized to 3p14.2 is altered in OSCC with decreased or aberrant protein [60] but no mutations and deletions [61,62]. The FHit protein has dinucleoside triphosphate hydrolase activity. However, the relationship between FHit activity and carcinogenesis of OSCC is still unclear.

4.2. Tumor suppressor genes

A number of downregulations of TSGs were closely related to the LOH in OSCCs. We describe here the representative three TSGs, TP73, APC, and CDKN2A.

4.2.1. TP73

Much evidence also suggests that TP73 acts as a TSG and is abnormally expressed in many human cancers. TP73 gene, with a significant homology to p53, was located just at the D1S468 locus of 1p36.3. Allelic loss of TP73 in 41 OSCCs had a frequency of 73% [15]. And also, TP73 was inactivated by the methylation-dependent silencing of this gene, and was associated with the tumor progression of human OSCC.

4.2.2. APC

APC is located on chromosome 5q21-q22 and is associated with the development not only of FAP but also of cancers in digestive organs. The role of APC in tumorigenesis is associated with intracytoplasmic accumulation of beta-catenin in response to carcinogenic stimuli, as the WNT signal. LOH of the APC was detected in 72.7% of OSCCs [11], and associated frequently with heavy smokers and drinkers, and in tumors with p53 mutations and human papilloma virus infection, suggesting that abnormalities in APC were closely related to the etiology and development of OSCC.

4.2.3. CDKN2A (p16)

The finding of frequent homozygous deletions in a wide variety of cancer cell lines focused attention upon the CDKN2A gene, a negative regulator of cell cycle progression located at chromosome 9p21. LOH of the CDKN2A was detected in 53% of OSCCs [8], and 2 of 50 (4%) OSCCs had nonsense mutations of the CDKN2A. It is unclear whether all of the allelic loss events at 9p21 in oral cancer are associated with CDKN2A inactivation.

4.3. Network and gene ontology analyses of genes located on LOH loci in OSCC

From 44 reports of OSCC-associated LOH, we extracted 28 downregulated genes in OSCCs (Table 1). We carried out network analysis on the 28 genes using the IPA tool to investigate if these genes interact biologically. Of the 28 genes, 27 were mapped to genetic networks as defined. These networks described functional relationships between gene products based on known interactions in the literature. Five identified networks were associated with the functions of cancer, cell cycle, cellular growth, and proliferation, etc. (Table 2). We also performed gene ontology analysis using the IPA tool. Five functions were identified as high-level functions (Table 3). Of them, the top function was the cancer-related function (Table 3, p = 8.77E−10 to 7.75E−03). Furthermore, to investigate the network of 19 cancer-related genes (Table 3), we performed network analysis using the IPA tool. Consequently, we found a major network (Fig. 2) including 13 cancer-related genes (Table 4). The shaded genes are those identified from previous OSCC-associated LOH studies, and the others are associated with the regulated genes based on network. These results indicated that carcinogenesis would be correlated with not only LOH but also networks of several cancer-related genes.

4.4. Comparison between LOH in OSCC and that in other SCCs

Some groups reviewed the LOH studies in other SCCs [1–17,53,56–60,63–86]. Here, we compared the status of LOHs between OSCC and other SCCs, including esophageal cancer, cervical cancer, and lung cancer. Similar to loci of LOH in OSCC, other SCCs demonstrated the relationship between LOHs and TSGs, such
as TP53, TP73, and RB1. Therefore, OSCC-specific LOH loci were not identified so far.

5. Conclusions

This review aimed to explore current knowledge about LOHs in OSCC, which develops after a long carcinogenesis cycle and multiple stages. A number of TSGs are involved in this process. As a result, allele loss chromosome regions, where candidate TSGs are located, were important in terms of the progress of OSCC. This summary of LOHs would be helpful for monitoring of clinical behaviors in OSCCs; e.g., we had revealed the relationship between the chromosome aberrant in OSCCs and their prognosis using circulating DNA in the peripheral blood [63].

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References


