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ORAL CANCER RESEARCH WITH AN EMPHASIS ON GENOMIC ANALYSIS

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Abstract

Ablative surgery has been a standard procedure for many years, but during the past few years there has been a significant improvement in our understanding of invasion and metastasis in oral cancer. Each procedure has to be planned individually. Before surgery, every attempt must have been made to arrive at a proper diagnosis, including the exact nature and aggressiveness of the tumor and an analysis of the patient’s genetic background. These procedures and the prognosis will be discussed.

Key words: Oral cancer—Epidemiology—Clinical features—Customized therapy—Genomic diagnosis

INTRODUCTION

The development of medicine and molecular biology has revealed that acquired gene failures cause cancers\(^{20}\). Oral cancers result from repetitive injury to the oral mucosa by external stimulation and multi-step changes in genes\(^{34}\). Injury to DNA by environmental oncogenic factors such as ozone, freon, ultraviolet rays, and environmental hormones also leads to oncogenesis\(^1\). The incidence of cancers will increase in Japan due to its rapidly graying society\(^{29}\).

The diagnosis and therapy of cancers have markedly progressed. More accurate diagnoses are possible before therapy, due to the development of medical engineering techniques such as helical CT (Computed Tomography),

An overview of this study was presented at the 22nd Asia Pacific Dental Congress (May, 2000, Tokyo)
MRI (Magnetic Resonance Imaging), ultrasonography and PET (Positron Emission Tomography).

The development of molecular biology has enabled the genetic diagnosis of cancers; the proteins produced by oncogenes are detectable and useful as new tumor markers and prognostic factors. Better operative techniques, including function-preserving surgery and reductive surgery, with less burden on patients and more treatment effectiveness have been devised\(^{21}\). In radiation therapy, in addition to the improvement of conventional methods, proton and heavy charged particle therapies have been introduced\(^{9}\). This progress has markedly influenced the patient’s quality of life (QOL).

In this paper, focusing on clinical diagnosis, therapy, and genetic analysis, we would like to discuss the present situation.

**EPIDEMIOLOGY OF ORAL CANCER**

Oral cancers are classified by location into carcinoma of the lip, buccal mucosa, gingiva, palate, floor of the mouth, tongue, central mandible, and maxillary sinus. The incidence of oral cancers analyzed in the First Department of Oral and Maxillofacial Surgery, Tokyo Dental College, is, in the order from highest to lowest, tongue, mandibular gingiva, floor of the mouth, buccal mucosa, maxillary gingiva, palate, and lip; these results are similar to those in other hospitals\(^{34}\) (Fig. 1). According to a report by the Japanese Ministry of Health and Welfare\(^{5,19}\), mortality per 100,000 people from oral and pharyngeal cancers was 2.4 in males and 1.3 in females in 1975, 5.1 in males and 2.9 in females in 1995, and it will be 8.6 in males and 5.2 in females in 2015, becoming three times higher\(^{5,29}\) (Fig. 2).
Generally, the incidence in males is approximately twice that in females and shows some differences in the site of predilection, gender, and age among races and regions. Mortality due to oral cancers between 1988 and 1992 was 2.3 in Japan, 6.4 in the United Kingdom, 13.2 in France, 4.6 in Australia, 3.7 in the United States, 14.5 in Singapore, and 15.6 in Hong Kong\(^5\) (Fig. 3). Although the incidences of oral cancers slightly differed in these reports, they are influenced by differences in race, life style, and environment\(^{19,27,28}\). For example, the incidence of oral cancer is high in male smokers, with a threefold higher incidence in patients with a smoking habit than in those without\(^{19,27}\). The relationship with drinking is also important; the concentration of alcohol, consumption level, starting age, period, and combination with smoking are risk factors for oral cancers\(^{19,28}\). Close relationships between the incidence of oral cancers and the habit of masticating chewing tobacco in India and Southeast Asian countries have been reported\(^5\). Oral cancers mainly occur in patients more than 50 years old, as do other cancers, but they also occur occasionally in patients as young as 20 years old\(^{29}\). An increase in patients in their twenties has recently been reported and requires careful observation. Because squamous cell carcinoma histologically accounts for more than 80% of oral cancers, this type of carcinoma will be the focus of the following discussion\(^{39}\).

**CLINICAL EXAMINATION**

It is well known that most oral cancers are visible and palpable, unlike malignant tumors in other organs, which is an advantage for their early detection\(^{25}\). However, early detection and early treatment do not always actually occur. This is because the macroscopic findings of early oral cancers are frequently markedly similar to those of benign tumors and other mucosal diseases. Furthermore, because the macroscopic clinical findings vary according to the site and stage, it is not easy to reach prompt and accurate clinical diagnoses only by palpation\(^{25}\).

1. **Morphological characteristics**

The macroscopic forms of squamous cell carcinoma that occur in the oral mucosa can be roughly categorized into three types; exophytic growth, endophytic growth, and

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Fig. 3 Incidence rate of oral cancer worldwide by sex\(^{19,29}\)
intermediate\textsuperscript{39} (Fig. 4). The exophytic growth type is further classified into papillary and granulomatous types, the endophytic growth type is further classified into ulcerative and swelling types, and the intermediate type is further classified into leukoplakie and erosive types\textsuperscript{30}. These forms of squamous cell carcinoma usually do not appear as one type alone, but as a mixture. The clinical appearance commonly changes from the initial phase to the final stage. There are no relationships between morphological form and grade of malignancy\textsuperscript{25}.

2. Chromatic analysis

Autofluorescence, toluidine blue vital staining, and iodine staining are usually used. In autofluorescence, fluorescent photographs are taken of the oral lesions, and the color of the films is evaluated using a chromameter\textsuperscript{15}. In cases of oral cancers, deep orange is detected. Since photography and specific apparatus such as the chromameter are necessary, this method is not generally used.

Recently, dye staining has been applied to diagnosing oral lesions\textsuperscript{27,36}. In this method, a specific dye is applied directly to the lesions to visualize early changes in the mucosal areas, which are not easy to see toluidine blue vital staining (Orascan) and the iodine reaction method are generally used\textsuperscript{60} (Fig. 5). In toluidine blue vital staining, the evaluation (oral cancer) zone is the area deeply stained by toluidine blue. This staining emphasizes the concavoconvexity of the lesions by the stagnancy of the staining liquid, making morphological observation easy. Macroscopically concavoconvex, irregular, ulcerative areas with abraded mucosal surfaces and tumors showing epithelial defective areas are detected as deeply stained (oral cancer) areas (Fig. 6).

In iodine staining, although the normal mucosa is stained black and brown, lesions are not stained, making differential diagnosis possible. The iodine glycol color-developing reaction occurring in the stratified squamous epithelium in the normal oral mucosa the basis of in this method\textsuperscript{6,27} (Fig. 7). Non-stained areas consist of pathohistologically highly heteromorphous epithelium, and carcinoma lesions \textit{in situ} also appear as non-stained areas (Fig. 8). The combined use of the two staining methods is also useful. Toluidine blue staining and iodine staining are frequently used as a double staining method in the oral cavity to determine the surgical resection margins and to select the best site for biopsy.

GENETIC ANALYSIS

Cancer studies have made remarkable progress along with the advance of molecular biology, and cancers are generally accepted as diseases due to genetic disorders. It has been reported that carcinogenesis of a cell involves the activation of more than two oncogenes and the inactivation of more than two tumor suppressor genes (TSG)\textsuperscript{1,35}. Nearly 40 TSG
1. Rinse with water and dry up
2. Apply iodine glycerin (10 to 20 seconds)
   - Iodine: 10 gm
   - Potassium iodine: 8 gm
   - Zinc sulfate: 1 gm
   - Glycerin: 35 ml
   - Distilled water: 65 ml
3. Wait for 1 to 2 minutes

Fig. 6 A 53-year-old male with squamous cell carcinoma of tongue. Clinical appearance before the staining (left). Appearance after the application of toluidine blue (right).

Fig. 7 Method of mucosa iodine staining

Fig. 8 A 70-year-old female with squamous cell carcinoma of tongue. Clinical appearance before the staining (left). Note the unstained area in a tongue lesion (right).
have been isolated as causative genes of genetic tumors and as genes that are probably inactivated in non-genetic tumors\textsuperscript{20}. There have been only a few reports of TSG in oral cancers, and only 10 genetic abnormalities have been investigated\textsuperscript{20}. It is considered that oral cancers do not develop by a malfunction of a sole specific gene, as is observed in other some cancers, but from a combination of several genes with complex relationships.

1. Tumor suppressor gene (TSG)

TSG related to oral cancers are WT, VHL, RB, APC, DCC, p16 and p18 (p16 family), p53, and p2\textsuperscript{3,4,7,18,32,40}. Furthermore, in the experimental model of oral cancers, the DOC1 gene (located in the long arm of chromosome 11) was isolated and identified as a completely new TSG of oral squamous cell carcinoma\textsuperscript{27,31,37,44}. Further evaluation of this gene in human oral cancers is expected\textsuperscript{38}. The discovery of fragile genetic structures in oral cancers such as genetic instabilities and scarcity or absent allelic genes can lead to the elucidation of new cancer-related genes\textsuperscript{8,11,33} (Fig. 9). Our department (Tokyo Dental College) has evaluated abnormal conditions in chromosome 2, 3 and 21, which have not been reported, using microsatellite markers.
by PCR-LOH analysis\textsuperscript{3,16,17}. Among the results of our analysis of 21q, 3 loci or absent regions on the long arm in chromosome 21 were commonly identified in oral squamous cell carcinoma, raising the possibility that three types of unknown TSG are present\textsuperscript{41} (Fig. 10). The ANA gene in chromosome 21 was also isolated and identified. Evaluation of the incidence of the ANA gene by the RT-PCR method showed markedly high percentages of absence or decrease\textsuperscript{42,43}. We also confirmed cases in which abnormal sizes of mRNA were identified. Since this gene has not yet been investigated in oral cancers, further analysis is expected.

2. DNA diagnosis

Genetic diagnosis and treatment technology applicable to oral cancers: The prediction of high-risk groups with high carcinogenicity, determination of high malignancy in cancers, diagnosis of prognosis and metastatic potential, sensitivity to chemotherapy and radiation, and determination of treatment methods are now possible. It is inevitable that new medical and ethical problems will result from the genetic diagnosis of cancers. For example, if a seemingly healthy person were diagnosed as being a hetero-carrier of genetic mutation by genetic diagnosis before the occurrence of disease or by genetic diagnosis of the risk of occurring disease, important medical information has been obtained, showing that diagnosis before the occurrence of disease is possible. However, it is difficult to determine how to deal with the results in diseases with low permeation rates of genetic mutation and in cases in which it is unclear when disease will occur. Furthermore, if such data are available to third persons, patients may be restricted in life and suffer discrimination. Therefore, it is important to evaluate the risk of having diseases and the advantages and disadvantages of treatment.

The following introduces the genetic diagnosis performed in our department. It has been reported that oral cancers are frequently combined with superior alimentary duct cancers. Smoking and drinking have been reported to be risk factors for combined cancers, so we investigated whether aldehyde dehydrogenase 2 (\textit{ALDH2}) and glutathion S-transferase M1 (\textit{GSTM1}) defective genotypes could be new risk analysis factors for combined cancers\textsuperscript{10}. These are both drinking and smoking-related genes common to oral and esophageal cancers. Performing DNA analysis of 191 patients with oral cancers by the PCR method, we found that \textit{ALDH2} deficiency was useful as a risk factor marker for combined cancers of the oral and superior alimentary duct (Fig. 11).

Recently, DNA chip technology has been developed to efficiently analyze total genetic function. Using this technology, newly arising genetic changes are detectable, and serial changes in the incidences of various genes and differences in the incidence patterns of several specimens can be examined simultaneously. By inputting the data obtained into a computer, a simulation modeled on a complex biological system can be performed. Furthermore, by investigating the sensitivity of patients’ genes, it is possible to use the data obtained for diagnosis and the selection of treatment methods. In the future, the concept of “functional genomics” will be important as a “post-genome” study goal. Therefore,

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\textsuperscript{*} p=0.05

Fig. 11 \textit{ALDH2} in relation to the risk for oral cancer in Japanese drinkers.
it will be important to make clinical use of analytical gene results.

TREATMENT

The treatment of oral cancers mainly consists of surgical treatment, radiotherapy, and chemotherapy and immunotherapy as auxiliary treatments. These treatment methods are used in combination, based on the size of the primary lesion, carcinogenic area, presence or absence of metastasis to the cervical lymph nodes, and histological malignancy.

Surgical treatment is preferred in advanced oral cancers with metastasis to the cervical lymph nodes. In cases of advanced tumors, suppression is impossible with only one treatment method, and the use of surgery, radiotherapy, and chemotherapy is frequently combined to improve the treatment effect.

1. Radiotherapy

For radiotherapy, external irradiation is usually selected, and radiotherapy is classified into preoperative and postoperative irradiation. The purposes of preoperative irradiation include: suppression of the growth and metastasis of cancers, prevention of cancer cell scattering during surgery, prevention of local recurrence, and reduction of the size of cancers to increase surgically indicated cases.
The purpose of postoperative irradiation is to supplement the technical and anatomical limitations during surgery and to increase the possibility of radical cure. The radiation dose is approximately 40 Gy for preoperative irradiation, and more than 70 Gy in the area in which the remaining tumor is most suspected for postoperative irradiation; this requires larger doses than does preoperative irradiation.

In cases of T1 and T2 oral cancers without metastasis to the cervical lymph nodes, internal radiation is useful (Fig. 12). The advantage of this therapy is that, because internal radiation is a local therapy, invasion of the whole body is less in comparison with surgery and chemotherapy, and it is possible to completely preserve morphology. The five-year survival rate is approximately 70% for T2 carcinoma of the tongue (Oral and Maxillofacial Surgery, National Tokyo Medical Center; Fig.
However, in cases of cancer cells infiltrating deep areas or large tumors the control rate of internal radiation is low. Furthermore, it has been reported that metastasis to the cervical lymph nodes is apt to occur after internal radiation. In selecting cases for radiotherapy, precise local and systemic diagnosis, and sufficient attention are necessary. Recently, the National Institute of Radiological Sciences presented heavy charged particle therapy as a new radiotherapy\(^9\) (Fig. 14). From the phase study, the normal tissue reaction was low, and the tumor reaction was slight at the end of irradiation, but there was almost complete disappearance six months after the end of irradiation\(^9\). Heavy charged particle radiation is excellent in physical dose distribution and has high biological effects, so a better treatment effect on tumors such as adenoid malignant tumors and malignant melanoma, which are incurable by conventional photon beam irradiation, is expected.

2. Chemotherapy

Chemotherapy is selected as an auxiliary therapy for oral cancers and also when surgery is rejected or impossible. Based on various mechanisms and basic data of, the concept of biochemical modulation has recently received attention\(^25\). This concept is to modulate the pharmacokinetics of main effectors by adding secondary drugs (modulators which are chemotherapy or non-chemotherapy drugs, that adjust the metabolic system of cancer cells, pharmacokinetics of effectors, and biochemical changes in metabolism in the target area and reaction site of effectors\(^12\)). By adding modulators, the anti-tumor effects inherent in effectors can be specifically enhanced, and adverse effects decreased. In principle, modulators themselves do not have either anti-tumor effects or adverse effects\(^13\). 5-FU and CDDP, 5-FU and MTX, 5-FU alone, or MTX alone as effectors are generally used in combination with leucovorin as a modulator. Chemotherapy using a drug cocktail is mainly based on clinical experience. We evaluated the differences in survival rate between patients with advanced T3 and T4 oral cancers who received preoperative chemotherapy and those who did not (Oral and Maxillofacial Surgery, Tokyo Metropolitan Komagome Hospital). The survival rate was significantly higher in patients who received preoperative chemotherapy, proving its usefulness (Fig. 15). It is necessary to increase the success rate of biochemical modulation to further improve patient survival and to improve their quality of life (QOL).
3. Surgical therapy

Surgery with excision is the most reliable method for treating oral cancers\textsuperscript{21}. However, because the oral cavity has important roles in speech, mastication, and deglutition, functional disorders become significant after surgery with excision. Due to the developments in pathological and surgical methods, it is now possible to completely excise tumors by methods appropriate for each tumor. Furthermore, it is also possible to minimize the excision range of normal tissue adjacent to tumors and to preserve oral morphology and function. This is because the pathological degree of differentiation and clinical tumor progression range can be precisely investigated, enabling secure excision\textsuperscript{30}. Furthermore, it is suggested that tumor infiltration into the bone is detectable by the presence of IL-6, which is a latent factor of tumor cells. Investigation into the presence of cytokines that are latent tumor factors and DNA diagnosis will be useful for evaluating the malignancy of oral cancer, and for surgery reduction.

Reconstruction surgery is performed for morphological and functional recovery after excision\textsuperscript{22,24}. In cases in which extensive excision was performed due to tumor progression,
reconstruction by microsurgery is recommended (Fig. 16), because it is useful for improving the three-dimensionally complex morphology in the oral cavity. At present, nerve grafting is performed simultaneously, enabling the improvement of sensory and kinetic functions.

In cases in which metastasis to the cervical lymph nodes occurs or later metastasis to the cervical lymph nodes is suspected, dissection of the cervical area is performed. In our department (Tokyo Dental College), functional cervical dissection is frequently performed, preserving the sternocleidomastoid muscle, accessory nerve, and internal jugular vein. Figure 18 shows the five-year survival rate of cases that underwent surgery. The cure rate is close to 70%, even in advanced cases with stage IV oral cancer.

4. Customized treatment

Genetic diagnosis obtained from the development of molecular biology allows the investigation of the individuality of each cancer. As a result, it may become possible to control all oral cancers by performing forestalling treatments appropriate for each cancer. With the developments in pathology, molecular biology, and surgical methods, the diagnosis and treatment of oral cancers have been established. Treatment results have improved, and patients can return to work sooner after treatment due to progress in reconstruction methods. However, even if the best treatment is performed based on state-of-the-art diagnosis, it is a fact that a certain percentage of oral cancers with high malignancy exist for which tumor control is impossible. At present, it is difficult to understand the pathologic conditions of those cases before surgery, as a result, they are recognized later, resulting in patient suffering. The conquest of oral cancers with high malignancy remains a challenge for the future. As a partial solution measure, the molecular biological approach, including genetics, is useful. The discovery of fragile genetic structures in oral cancers, such as genetic instabilities and the scarcity or absence of allelic genes, can lead to the elucidation of new cancer-related genes, so DNA diagnostic methods will become more useful for oral cancers. It is important to simultaneously perform both pathologic diagnosis and DNA diagnosis as routine examinations to detect many cancer cases (Fig. 19). The age is coming in which those results will be used to select treatment methods appropriate for each cancer by understanding its specific characteristics. Customized treatment will not only be possible but also necessary in the future.

We believe that the conquest of oral cancers as well as other cancers is possible in the twenty-first century.

REFERENCES

Japan.


