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Evaluation of Frozen Section Diagnosis and Rapid Intraoperative Flow Cytometric Analysis of Oral and Maxillofacial Lesions

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Abstract Frozen section diagnoses of oral and maxillofacial lesions were performed in 60 cases at the Yamaguchi University Hospital over the course of 3 years. Frozen section diagnoses were mainly used to determine the surgical margins (68.3%) and/or lymph node metastasis (71.7%). Only 20% of those frozen sections were used to obtain pathological diagnoses of lesions, which were uncertain diagnoses before the operation. The average number of frozen sections per operation was 4.4, with 14 being the most. Ninety-eight percent of the frozen section diagnoses were consistent with the final permanent section diagnoses. In some of these cases, a rapid measurement of DNA patterns, using flow cytometry (FCM), was performed. The incidence of DNA aneuploidy was in 57.1% of squamous cell carcinoma and in 80% of malignant salivary gland tumors. In contrast, DNA aneuploidy could not be found in benign lesions. Where DNA aneuploidy was found, we almost always diagnosed that section as to be malignant. Therefore, the measurement of DNA ploidy patterns would be useful in supporting the frozen section diagnoses.

Key Words: Frozen section diagnosis, Flow cytometry, DNA aneuploidy

Introduction

Frozen section diagnosis during the operation has confidently been used in many hospitals. The chief objective of a frozen section diagnosis is to allow an immediate decision regarding surgical therapy. Not only may further surgery be indicated, but the type and extent of surgery can depend on the pathologist's report. Frozen section diagnosis has been accurate for specific pathologic diagnosis, however, diagnostic mistakes have occurred in a few cases, resulting in unnecessary surgery for patients[1-4]. In order to prevent these mistakes, we should have a better understanding about the frozen section's peculiarities. To support the frozen section diagnosis, a rapid DNA pattern analysis by flow cytometry (FCM) has been used recently[5]. On the principle that DNA aneuploidy often appears in malignant tumors, whereas not in benign tumors or benign lesions[6,7], it would be useful to assist the frozen section diagnosis.

The purpose of this study is to evaluate the accuracy of frozen section diagnosis and to

examine the effect on the outcome of the patient's treatment. And also, to evaluate the application of DNA ploidy pattern diagnosis for oral and maxillofacial lesions in our hospital.

Materials and Methods

Frozen section diagnosis was performed in 60 cases of oral and maxillofacial lesions at the Yamaguchi University Hospital from 1986 to 1989. Frozen sections were prepared on a cryostat, using disposable knife blade, and stained rapidly with hematoxylin and eosin. Specimens were reviewed by pathologists.

Twenty-five cases were subjected to rapid measurement of DNA patterns using FCM, a technique which were previously reported[5]. In brief, small pieces from the resections were washed in cold phosphate-buffered saline (PBS) and minced with scissors. A suspension of isolated nuclei was prepared with 0.2% Triton X in PBS used as a detergent. After addition of 0.1% RNase, the suspension was filtered through a 47 μ nylon mesh and stained with propidium iodide (PI). The DNA content was measured with a FACS Analyzer (Becton Dickinson Co., Mountain View, USA) that was calibrated using fluorescence beads so that the coefficient of variation (CV) was less than 2%. A total of at least 10, 000 cells were analyzed per case. DNA ploidy was determined according to the principle, reported by Sasaki and his co-workers[8].

Results

Patients ranged in age from 26 to 87, with an average age of 59.7 years. Preoperative diagnoses were as follows: Forty-nine out of 60 cases (81.7%) were malignant tumors diagnosed by biopsy. Forty-six of these 49 cases (93.8%) were histologically diagnosed as squamous cell carcinomas. There were also 2 cases of benign tumor. Furthermore, the cases of unknown diagnosis, where a biopsy was not performed before the operation, consisted of; 3 malignant salivary gland tumors, one case of lymphoma, lymph adenitis, ameloblastoma, radicular cyst, granulation tissue and a normal lymph node diagnosed using frozen section. Of the 60 cases, which used intraoperative frozen section diagnosis, operations for malignant tumors were perfomed in 53 cases of them (84.1%). Operations for benign tumors and begin lesions were performed in 2 and 5 cases, respectively. Of the specimens taken, they were divided into 3 types (Table 1). Specimens for surgical margins were used for examining the extent of lesions. Lymph node specimens were used for examining the invasion of regional lymph node. Specimens for characterization were used in diagnosing surgical resections, which were uncertain diagnoses before the operation. Forty-three of 60 cases (71.7%) were used in detecting the extent of the lesions and 41 of 60 cases (68.3%) were used in detecting the invasion of lymph node metastasis. Only 12 of 60 cases (20.0%) were used in detecting the characterization of the lesions.

Table. 1 Purpose and number of intraoperative frozen section diagnosis

Purpose	No. of cases
Surgical margin	43 (71.7%)
Lymph node	41 (68.3%)
Characterization	12 (20.0%)
Total	60

According to the regional differences, the number of specimens used for intraoperative frozen section diagnosis were shown in table 2. Highest number of specimens per operation was found in maxillary carcinoma being 6.6 average spcimens with a range of 3-14. The specimens mainly consisted of lymph nodes (mean number of 4.8 specimens). In the case of mandibular, tongue and oral floor carcinoma, the number of specimens were more prevalent within the surgical margin than the lymph nodes. Frozen section diagnosis for characterization were performed in all cases of benign lesions and in a few of the malignant tumors. Respectively, the average number of frozen sections per operation was 4.4, with 14 specimens being the most per operaton.

Five out of the 60 cases that underwent frozen section diagnosis benefited from this process because; we gained added information if we needed to extend the resection beyond the original line at the initial proce-

Table 2 Cases of intraoperative frozen section diagnoses

Diagnoses (No.)	No. of specimens			Characterization
	Surgical margin	Lymph node	Total no. (Range)	
Maxillary carcinoma (9)	1.8	4.8	6.6 (3-14)	1 case
Mandibular carcinoma (13)	2.5	1.6	4.1 (1-9)	1 case
Tongue and oral floor carcinoma (19)	3.2	2.2	5.4 (1-10)	none
Lip or buccal carcinoma (4)	1.7	2.3	4.0 (3-5)	none
Salivary gland carcinoma (6)	1.5	3.3	4.8 (1-5)	3 cases
Other malignant tumors (2)	0	0	0	1 case
Benign lesions (7)	0	0	0	7 cases
Total average	2.0	2.4	4.4 (1-14)	

Table 3 The frequency of DNA aneuploidy

Histological diagnosis	DNA distribution		Total
	Diploid	Aneuploid	
Squamous cell carcinoma	6	8	14
Adenoid cystic carcinoma	1	2	3
Mucoepidermoid carcinoma	0	2	2
Pleomorphic adenoma	2	0	2
Ameloblastoma	1	0	1
Granulation	1	0	1
Normal lymph node	1	0	1
Radicular cyst	1	0	1

dure. In two patients, the operative procedure was extended from partial neck dissection to total neck dissection, and in one patient, the initial procedure was extended to total tongue excision. One patient with squamous cell carcinoma of buccal mucosa underwent a wide excision, including excision of parotid gland after ductal invasion was demonstrated by intraoperative frozen section diagnosis. One patient with bilateral maxillary sinus carcinoma, determined in preoperative diagnosis, underwent dissection of only the lateral maxillary bone owing to the indication from intraoperative frozen section diagnosis.

Fifty-nine of 60 cases (98.3%) were in complete agreement between intraoperative frozen section diagnosis and the final permanent section diagnosis. In one case of a salivary gland tumor, a benign neoplasm was suspected on frozen section evaluation. The pathologist however did not rule out the possibility of malignancy, and deferred to permanent section diagnosis. In this case, on the basis of the intraoperative diagnosis, a subtotal parotidectomy was performed without seventh-nerve resection. The permanent section diagnosis was a well-differentiated, mucoepidermoid carcinoma. The lesion was entirely removed as examined by permanent sections. Post operative radiation and chemotherapy were administered and the patient has had no recurrence for 2 years after surgery.

The frequency of DNA aneuploidy in various cases of oral and maxillofacial disease

was shown in Table 3. Twenty-five out of 60 cases, which were diagnosed by frozen section, could be measured by FCM. In squamous cell carcinoma, the incidence of DNA aneuploidy was 57.1% (8 of 14 cases). In malignant salivary gland tumors, such as adenoid cystic carcinoma and mucoepidermoid tumor, the percentage of DNA aneuploidy was 80.0% (4 of 5 cases). In contrast, DNA aneuploidy could not be found in the benign lesions, such as pleomorphic adenoma, ameloblastoma and granulation tissue. Typical DNA histogram patterns are shown in Fig. 1. In cases of squamous cell carcinoma and mucoepidermoid carcinoma, FCM analysis of these sections showed DNA aneuploidy patterns, which indicated malignant tumors. However, some cases of squamous cell carcinoma did not show a DNA aneuploidy pattern. In a case of pleomorphic adenoma, FCM analysis of this section showed a DNA diploid pattern, which indicated the possibility of a benign lesion.

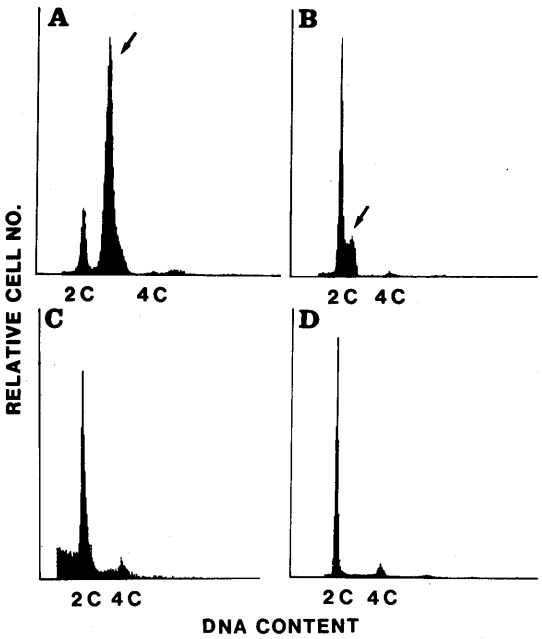

Fig. 1 Four cases of the typical DNA distribution in oral and maxillofacial diseases. A: A case of squamous cell carcinoma of maxilla diagnosed by a frozen section. FCM analysis of this section shows a DNA aneuploidy pattern (arrow), which indicates a malignant tumor. B: A case of well differentiated mucoepidermoid carcinoma of submandibular gland. A DNA aneuploidy pattern (arrow), which is different type from Fig. 1-A, also indicates a malignant tumor. C: A case of squamous cell carcinoma of the tongue. Although malignant tumor was diagnosed by frozen section, FCM analysis do not show aneuploidy. D: A case of pleomorphic adenoma of parotid gland. FCM analysis of this section shows a DNA diploid pattern, which indicates the possibility of a benign lesion.

Discussion

In general, the purpose of intraoperative frozen section diagnosis are to detect the following: (a) extent of the lesions, (b) invasion of lymph node metastasis, (c) characterization of lesions which were uncertainly diagnosed before an operation. Iri and his co-workers[1] reported that frozen section diagnosis was frequently used for detecting the characterization of lesions. Eighty-five percent of their 750 cases that underwent frozen section evaluation at the Section of Surgical Pathology of their hospital were used for this purpose. In contrast to this report, in our hospital, specimens for frozen section diagnosis in oral and maxillofacial lesions were mainly used in discerning whether cancer cells in the surgical margin and/or lymph node metastasis were present or not. The reason for their being only a few cases of characterization is due to the ease of diagnosis before surgery. That is, it is easy to make a diagnosis by biopsy because of the access to the surface and high visualization of lesions in oral and maxillofacial diseases. Those of salivary gland diseases are the exception. Moreover, resected lesions were limited because of aesthetics and functional aspects involved in oral and maxillofacial diseases. Therefore, it would be especially important to examine the extent or invasion of the lesions.

Hillel et al.[3] reported that 75 of 108 resections (69%) were examined for frozen section diagnosis in parotid gland operation and

that 4 patients benefited by further surgery during the initial procedure. Rigual et al.[4] reported in a retrospective review of 100 patients wth salivary gland neoplasms, of those who received frozen section diagnosis, 12 patients benefited by more extensive surgery during the initial operation. In our study, 5 of 60 cases (8.3%) benefited by further surgical proceedings during the initial operation. Four patients underwent wide excisions, and one patient a narrow excision.

FCM was applicable for DNA analysis in human tissue and provided relatively rapid and objective methods for detection of DNA aneuploidy[6,7]. It was usually performed within 15 minutes. Therefore, each time, preparation of both frozen section and FCM analysis, showed no difference. Moreover, DNA aneuploidy often appears in malignant tumors, whereas not in benign tumors or benign lesions[6,7]. Where DNA aneuploidy was found, we almost always diagnosed that section as to be malignant. Murakami et al.[5] reported on the frequency of DNA aneuploidy in various kinds of human tumors and concluded that; the measurement of DNA ploidy patterns could be useful in supporting the frozen section diagnosis, especially for the intraoperative diagnosis thyroid neoplasms.

With this in mind, 25 cases of oral and maxillofacial leisons, where frozen section diagnosis was performed, were simultaneously analysed with FCM in order to assist the diagnosis in this study. The frequency of DNA aneuploidy was 57.1% in the cases of squamous cell carcinoma and 80% in malignant salivary gland tumors, and DNA aneuploidy was not found in benign lesions. For the pathologists, intraoperative frozen section diagnosis would not be complicated in the cases of squamous cell carcinoma, however, it sometimes is difficult in the cases of salivary gland tumors. The accuracy of frozen section diagnosis in salivary gland neoplasms was reported 95%[3], 92%[4], with false -negative results occurring in only a few cases. To prevent these mistakes, it would be effective to simultaneously measure DNA ploidy pattern. The diagnosis of malignancy by frozen section would be supported by the appearance of DNA aneuploidy.

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