Effect of Surgical Instruments on Cytological Features: A Pilot Study of Mouse Tongue Cancer Models

Chieko ITOHARA, Katsumi HIDESHIMA, Joji SEKINE
Department of Oral and Maxillofacial Surgery, Shimane University Faculty of Medicine, Izumo, 693-8501, Japan
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Rapid intraoperative cytology is a common method for examining resection margins in cancer surgery. Cytopathologists often face challenges with cytological diagnoses because of cell degeneration by electric scalpels. This pilot study sought to elucidate the effect of surgical instruments on cytological features.

Mouse models of tongue SCC were used（n = 10）. Tumor was excised using a stainless-steel scalpel（n = 2, SS）, electronic scalpel（n = 2, ES）, or ultrasonic scalpel（n = 2, HS）. Imprint cytology specimens from resected tumor were subjected to Papanicolaou（Pap）or Giemsa staining. Remaining tissue was stained with hematoxylin and eosin. In SS specimens, heat-induced cell degeneration was observed less extensively than in ES. In perioperative rapid intraoperative cytology examination, surgeons should consider the possibility of changes in cellular morphology depending on the type of surgical instrument used. The cytopathologist should also be informed about the instrument used to resect the specimens.

Key words: surgical scalpel, oral cancer, rapid intraoperative cytology, cytopathy

INTRODUCTION

In surgical resection for cancer, it is important to resect the lesions with sufficient safety margins in order to prevent local recurrence［1-6］. Intraoperatively, frozen sections are subjected to routine histological examination to verify tumor-free resection margins. Although its diagnostic accuracy has been improved［7, 8］, this conventional histological method examines only some parts of the resection margins and is therefore not appropriate for surgery like hemiglossectomy, where an excision method that covers all the resection margins is required.

Recent studies in gastroenterological, urological, and mammary surgery have shown the utility of rapid intraoperative cytology in complementing diagnosis based on frozen tissue sections or accurately and thoroughly evaluating resection margins and sentinel lymph nodes［9-11］. Another study reported the utility of cytological specimens for accurately evaluating resection margins after surgical resection of lesions in different areas such as the oral cavity, the head and neck, ovary, lung, breast, and stomach［12-18］.

In rapid intraoperative cytology, the rapid Giemsa staining method is used to shorten the diagnostic time［19］. However, recent studies have developed a rapid staining method that uses Pap stain and air-dried specimens, enabling a substantial reduction in staining time while producing transparent stains as obtained from a regular Pap stain［20, 21］.

The primary site immediately after hemiglossectomy is shown in Fig. 1A. Blood on the surface of resected specimens was removed using saline and wiped using gauze（Fig. 1B）; then, the entire resected area of the specimen was imprinted onto a slide glass for cytological examination（Fig. 1C）［22］.

The ES causes the tumor cells to undergo liquefaction and cytoplasmic changes（Fig. 2A）due to heat generated by the electric current. This affects the tissue and cellular morphology, and so the fea-

Corresponding author: Joji Sekine, D.D.S. Ph.D, FIBCSOMS
Department of Oral and Maxillofacial Surgery, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan
Tel: +81-853-20-2301
Fax: +81-853-20-2299
E-mail: georges@med.shimane-u.ac.jp

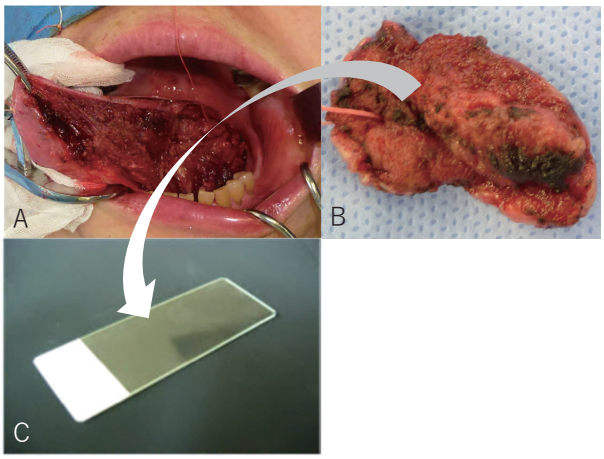

Fig. 1. Rapid intraoperative cytology
A: Primary site immediately after hemiglossectomy. B: Resection specimen. C: The entire resection area can be screened by pressing and imprinting the specimen onto a slide for cytological examination.

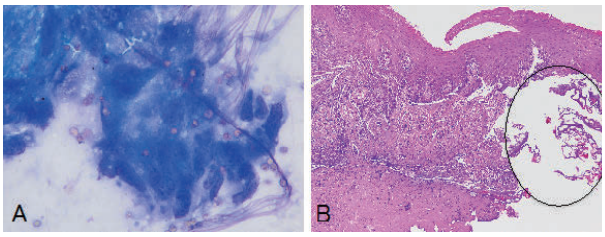

Fig. 2. Cytological and histological findings showing the effect of use of electronic scalpel
A: Tumor cells with ill-defined cell membrane due to thermal damage. Cell morphology (Giemsa stain, 100× magnification). B: Heat-induced degeneration and colliquation shown in the area enclosed by a circle. Tissue morphology (HE stain, 10× magnification).

tures are not well defined (Fig. 2B). Therefore, we conducted this pilot study using mouse models of tongue cancer, to clarify the effect of various surgical instruments on cell morphology. The animal protocol was approved by the local animal ethics committee of Shimane University Faculty of Medicine in 2010 (approval no. IZ21-153; date, November 20, 2010).

METHODS

Animal models and surgery

Mouse tongue cancer models were generated using 5-week-old male C3H/HeNCrl mice (n = 10) [23]. NRS-1 mouse squamous cell carcinoma (National Institute of radiological Sciences, Chiba, Japan) was transplanted to the back of 4 mice under general anesthesia (GA) with intraperitoneal injection of 50 mg/kg of pentobarbital sodium. After 4-5 days, the tumors grew to approximately 7×7 mm in size; the mice were euthanized under GA with intraperitoneal injection of 300 mg/kg of pentobarbital sodium, and the tumor masses were resected from the back. Resected tumors were then implanted to the tongue of 6 other mice under GA with intraperitoneal injection of 50 mg/kg of pentobarbital sodium. Five to 6 days after implantation, the tongue tumors were examined. These mice were subsequently euthanized under GA with intraperitoneal injection of 300 mg/kg of pentobarbital sodium.

A stainless-steel scalpel (#11, Feather Safety Razor Co., Ltd., Osaka, Japan; SS group, n = 2), an electronic scalpel (Coltène Whaledent Inc., Cuyahoga Falls, OH; ES group, n = 2), or an ultrasonic knife (hook-spatula type blade, Harmonic®, Johnson & Johnson Inc., Somerville, NJ; HS group, n = 2) were used to cut the intermediate part of the tumor (white arrow) (Fig. 3A).

The output of the ES was set to the minimum required. The initial output level was 3 (cut: 30; coagulation: 30) based on routine surgical practice.

In the HS group, output levels were increased for speedy incision and we reduced the output level of the generator to achieve a coagulation effect and the maximal output level was set beforehand to level 5. Also, we increased the output levels for faster incision and reduced the output level of the generator to achieve a coagulation effect, and the maximal output level was set beforehand to level 5. Furthermore, we set coagulation hemostasis and vascular exposure to level 3 as is used in daily surgical practice (Fig. 3B).

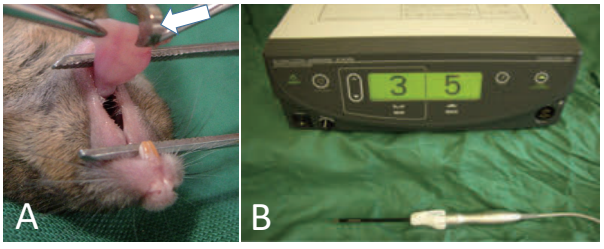

Fig. 3. Resection of tongue cancer using an ultrasonic scalpel
A: Surgical resection of murine tongue cancer by using ultrasonic blade (white arrow: hook type blade). B: The output for the ultrasonic scalpel was comparable with that used in routine clinical practice.

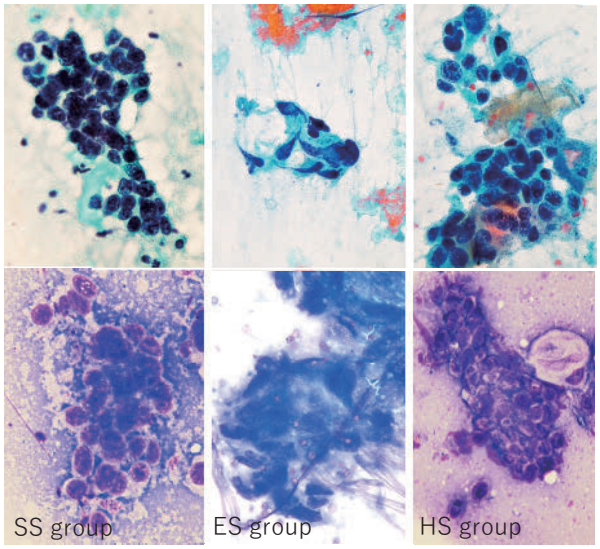

Fig. 4. Findings of imprint cytology
In the SS group, the nucleus and cytoplasm of individual tumor cells could be observed clearly because there was no cell degeneration. In the ES group, colliquation of the cytoplasm was observed. In the HS group, identification of nuclear architecture difficult due to degenerative changes observed in the nucleus of some tumor cells, although not as extensive as in the ES group. Upper panels, Pap staining; lower panels, Giemsa staining, 100× magnification.

Cytological and histological tissue preparation

The resection margin of the tumor was pressed onto a slide glass, and the slides were stained using the Pap or Giemsa method. Post-imprint tumor tissue was fixed in formalin and was processed into 4-μm sections for hematoxylin and eosin (HE) staining and evaluated histopathologically.

RESULTS

Cytological findings in Pap and Giemsa staining

In the SS group, cell clusters were observed against a necrotic background with dense cytoplasm and clear nucleoli; the nuclear/cytoplasmic ratio (N/C ratio) was high and the nucleus was round or oval. These hyperchromatic cells were identified as malignant tumor cell arising from squamous epithelium.

In the ES group, cells thriving in a hemorrhagic and necrotic background had a prominent nucleolus, suggesting that they were malignant. However, in many cells, the intranuclear structures as well as the cytoplasm had undergone colliquation and leakage.

In the HS group, although tissue structure was recognizable, identification of intranuclear structures was sometimes difficult due to eosinophilic coagulation in some parts. These findings suggest that ultrasonic wave frictional heat influenced the heat-induced denaturation. These cells had a high N/C ratio and pyknotic chromatin, and some multinucleate giant cells, suggesting that they were malignant tumor cells originating from the squamous epithelium. However, a group of cells displayed dense nuclei with irregular contours, presumably due to the colliquation of the cytoplasm (Fig. 4).

Histological findings in sections

The cross-section was sharp in the SS group, whereas in the ES group, colliquation of connective tissue as well as thin layers of degenerative tissue lacking structural definition was observed. Identification of the nucleus was difficult in some cells due to severe degeneration.

In HS group, there was tissue damage with eosinophil-induced coagulation. Thermal denaturation of protein seems to have been caused by ultrasonic heating, which is caused by ultrasonic vibrations. Nuclear structure was slightly difficult to see, but tissue architecture was recognizable (Fig. 5).

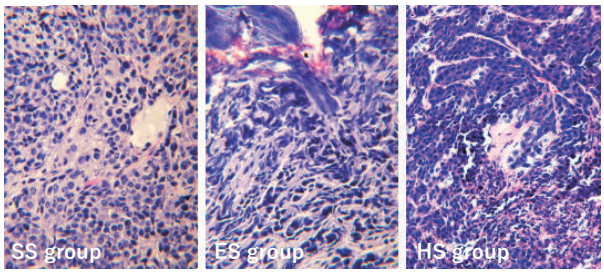

Fig. 5. Histological findings
In the SS group, the cross-section was sharp. In the ES group, colliquation of the cytoplasm and nucleus was observed in cells across the entire cross-section.
In the HS group, tissue structure was recognizable, but eosinophilic coagulation and cytopathy were observed in some parts (HE stain, 40× magnification).

DISCUSSION

In this pilot study, the ES and HS output was set at level 3, which is routinely used in clinical procedure. However, heat appeared to cause cytopathy in both the ES and HS group. Hambley *et al.* used stainless-steel scalpel, electronic scalpel, ultrasonic

scalpel, and CO_2 laser to make an incision through the skin on the back of pigs and sutured the wound [25]. After evaluating chronological changes in the healing process and wound closure tension, they reported that use of a stainless-steel scalpel contributed to the least tissue damage and fastest recovery time [25]. In addition, an incision made with an ultrasonic scalpel resulted in less tissue damage and faster recovery than one made with an electronic scalpel or CO_2 laser, suggesting that the use of ultrasonic scalpels is beneficial in terms of tissue healing [25]. Electronic scalpels separate and coagulate tissue by generating high-frequency waves that heat-denature proteins in the tissue. In contrast, ultrasonic scalpels cut tissue by propagating mechanical energy strong enough to break hydrogen bonds, and frictional heat generated at that time denatures and coagulates proteins [25, 26]. Thermographic analysis showed that ultrasonic scalpels did not carbonize the tissue or heat the tissue to above 80°C [26].

We previously investigated the engraftment of split thickness skin grafts to surgical wounds made by electronic or ultrasonic scalpels [27, 28]. The results showed that partial glossectomy performed with an electronic scalpel caused tissue damage even in the deep layers, whereas tissue degeneration was localized only on the resection surface after partial glossectomy performed by an ultrasonic scalpel [28]. These findings support the report by Hambley *et al.* [25], suggesting that clinical healing of a surgical wound made by the 3 types of surgical instruments used in this pilot study do not differ significantly.

The surgeon's choice of surgical instrument depends on the purpose. In our pilot study, the SS blade cut sharply, and the maintenance of cellular integrity was good, but bleeding was common. On the other hand, in the ES and the HS, there was no bleeding, but there was cellular damage.

However, ultrasonic scalpels affect cytopathy due to heat-denaturation, although not as extensively as electronic scalpels. When performing rapid intraoperative cytology, it is important to remember that these different surgical scalpels cause cell degeneration. Moreover, when performing surgery, it is important to clearly inform the cytopathologist about the type of surgical scalpel used.

In the case of bone tissue specimens, rapid histopathologic diagnosis is difficult because bone is not easily cut to prepare frozen sections, though cytology is an option. So, rapid intraoperative cytology is necessary [29].

In clinical cases, the problems are twofold. First, it is difficult to determine the definitive diagnosis because of cellular degeneration from damage caused by the surgical instrument. Second, routine rapid intraoperative cytology must be performed carefully, particularly to avoid inadvertent transfer of cancer cells to other sites. The procedure should be improved to solve these problems in the future.

In conclusion, it is important to inform the cytopathologist as to what kind of instrument was used to resect the specimens.

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