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Original Article

Comparisons of histological features among primary oral squamous cell carcinomas before and after adjuvant chemotherapy and their lymph node metastatic cancer lesions after adjuvant chemotherapy

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KEYWORDS
Squamous cell carcinoma;
Adjuvant chemotherapy;
Necrosis and degeneration;
Drug-induced squamous and

Abstract *Background/purpose:* Adjuvant chemotherapy has been used to control the primary oral squamous cell carcinoma (OSCC) size prior to surgical excision of the cancer. This study aimed to explore the histological changes of primary OSCCs and their cervical lymph node metastatic cancer lesions after chemotherapy.
Materials and methods: Thirty-three OSCC patients with eleven having cervical lymph node metastases received adjuvant chemotherapy before surgical excision of their cancer lesions. Hematoxylin and eosin-stained tissue sections of incisional biopsy, surgical excision, and cervical lymph node metastatic cancer lesion specimens were compared microscopically to observe the histological changes in the cancer tissues after chemotherapy.

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keratinizing metaplasia;
Epidermoid cyst-like lesion

Results: Common histological features could be found in the primary OSCCs and their cervical lymph node metastatic cancer lesions after chemotherapy. These included direct killing of cancer cells by chemotherapeutic agents, resulting in cancer cell necrosis and degeneration in the early phase, and squamous and keratinizing metaplasia of drug-induced cancer cells, leading to individual cell keratinization and keratin pearl formation in the later phase. There were also small nests of drug-resistant proliferating cancer cells in the inflamed fibrous connective tissue stroma. The most characteristic histological feature in the metastatic lymph nodes after chemotherapy was the keratinizing metaplasia of the metastatic cancer cells, resulting in the formation of epidermoid cyst-like lesions.
Conclusion: Although the cancer reduces its size after chemotherapy, residual cancer cells are consistently present in the primary OSCC lesions after chemotherapy. Therefore, wide surgical resection of the cancer is still needed to ensure the complete removal of all cancer tissues.

Introduction

Squamous cell carcinoma (SCC) is the most common malignant tumor in the oral cavity and it can be found in any sites of the oral cavity.[1,2] Oral SCC (OSCC) ranks as the sixth most prevalent cancer across both sexes and represents the fourth most common cancer for men in Taiwan.[3] Surgical excision with or without radiotherapy has long been the conventional therapeutic modality for OSCCs.[4]

In the last five decades, chemotherapy has been used as an adjuvant treatment for OSCCs prior to the surgical excision of the cancer in order to improve the resectability of large OSCCs and the survival rate of the OSCC patients.[4–8] In some institutions, including the Department of Dentistry, National Taiwan University Hospital (NTUH), adjuvant chemotherapy has been frequently included in the treatment protocol for previously-untreated OSCC.[7] It has been proved that chemotherapy can do so by exerting certain cytotoxic effects on the cancer cells to reduce the OSCC size. However, the mechanisms involved in this process remain unexplored. In order to explore the cancer cell killing process by chemotherapeutic agents, we tried to study the histopathological changes of the primary OSCCs and their cervical lymph node metastatic cancer lesions after adjuvant chemotherapy.

In this study, each OSCC patient with previously-untreated OSCC received adjuvant chemotherapy prior to the surgical resection of the cancer after the definitive diagnosis was established by incisional biopsy. It is therefore possible to compare the histological features of the previously-untreated primary OSCCs seen in the incisional biopsy specimens with the drug-induced OSCC tissues seen in the surgical excision primary OSCC and cervical lymph node metastatic cancer lesion specimens. The difference in histopathological features between the incisional biopsy and surgical excision specimens was attributed to the adjuvant chemotherapy. Although this study was focused on the histopathological changes of the primary OSCC tissue after adjuvant chemotherapy, it appeared that the cervical lymph node metastatic cancer lesions from primary OSCCs, when present, showed more obvious histological changes than their primary OSCC counterparts after adjuvant chemotherapy. Therefore, this study also tried to compare the drug-induced histological changes seen in the cervical lymph node metastatic cancer lesions with those seen in their primary OSCC counterparts.

The present study used hematoxylin and eosin-stained tissue sections to compare the histological features of incisional biopsy specimens of primary OSCC with those of surgical excision primary OSCC specimens and their cervical lymph node metastatic cancer lesion specimens after adjuvant chemotherapy.

Materials and methods

Study subjects

Thirty-three OSCC cases (from 28 men and 5 women) were retrieved from the files of the Division of Oral Pathology and Diagnosis, Department of Dentistry, NTUH during the period from 1992 to 2000. For each eligible patient, the following conditions were confirmed: (1) the clinical records of the original OSCC were complete and the patient did not receive any cancer-related treatments before; (2) there was an incisional biopsy specimen for the initial diagnosis of an OSCC; and (3) the patients received adjuvant chemotherapy before the surgical resection of their primary OSCCs. Of the 33 OSCC patients, 11 had concomitant cervical lymph node metastases but only 7 had their cervical lymph node specimens collected in this study. Therefore, these 7 OSCC patients had the specimens of both primary OSCCs and their cervical lymph node metastatic cancer lesions. The clinicopathological parameters of 33 OSCC patients are shown in Table 1.

Hematoxylin and eosin-stained tissue sections

We collected formalin-fixed paraffin-embedded tissue blocks of the 33 eligible OSCC patients mentioned above from the Department of Pathology, NTUH. For these OSCC patients, the hematoxylin and eosin (H&E)-stained tissue sections were prepared from formalin-fixed and paraffin-embedded tissue blocks of the incisional biopsy specimens before

Table 1 Clincopathological parameters of 33 oral squamous cell carcinoma (OSCC) patients in this study.

Clincopathological parameters	Patient number
Gender	
Male	28
Female	5
Age (year)	
≤39	6
40-59	17
≥60	10
Cancer location	
Buccal mucosa	16
Gingiva	7
Tongue	6
Lip	1
Soft and hard palate	2
Floor of mouth	1
Cervical lymph node metastases	
With	11
Without	22

chemotherapy, the surgical excision primary OSCC specimens after chemotherapy, and the cervical lymph node metastatic cancer lesion specimens after chemotherapy. These H&E-stained tissue sections were collected for histopathological examination in the Laboratory of Oral Histology and Oral Pathology, School of Dentistry, National Taiwan University. The histopathological changes of the primary OSCCs and their cervical lymph node metastatic cancer lesions after adjuvant chemotherapy were recorded.

Study limitations

Since this was a retrospective study with no prospective control, wide variations in the original OSCC status and different protocols of adjuvant chemotherapy were found from case to case. Therefore, comparisons were only made in each patient to understand the drug-induced histological changes seen in the specimens of primary OSCCs and their cervical lymph node metastatic cancer lesions after chemotherapy. No attempts were made to assess the differences in drug-induced histological changes among all OSCC specimens.

Results

In this study, 33 OSCC patients (28 men and 5 women) were included. The mean age of 33 OSCC patients was 52.8 years (range 29–84 years). In terms of OSCC locations, buccal mucosa was the most common site (16 cases), followed by the gingiva (7 cases) and the tongue (6 cases). Eleven of the 33 OSCC patients had concomitant cervical lymph node metastases (Table 1).

The incisional biopsy specimens of the primary OSCCs before chemotherapy showed a wide variation of histological features that included well-, moderately- and poorly-differentiated SCCs. When the incisional biopsy specimen included the cancer tissue and adjacent oral mucosa, a transition from normal to dysplastic oral epithelium and further to the SCC tissue could be found, and there might be a demarcation between tumor mass and adjacent normal or dysplastic oral epithelium (Fig. 1).

In surgical excision primary OSCC specimens removed from OSCC patients after chemotherapy, epithelial atrophy and surface ulceration were the common findings after chemotherapy. In addition, immediate or relatively long-term effects of chemotherapeutic drugs on the changes of histological features of OSCC could also be observed. The immediate drug effect on the OSCC tissue was presented in the cases operated soon after the cessation of chemotherapy. In these cases, some residual cancer cells of primary OSCCs showed cell necrosis and degeneration, and thus a large amount of cell debris and keratin materials could be found in the edematous stroma with both acute and chronic inflammatory cell infiltrates. At the edematous connective tissue stroma surrounding residual cancer cell debris and keratin materials, there might be macrophages and multinucleated foreign body giant cells executing phagocytosis of necrotic cancer cell debris and cancer cell-produced keratin materials.

The relatively long-term drug effects on the OSCC tissues were discovered in the OSCC cases resected later than 2 weeks after chemotherapy. In these cases, the chronic inflammatory reaction with stromal fibrosis could be observed. Moreover, in these surgical excision primary OSCC specimens, residual cancer cells were always found in the inflamed fibrous connective tissue stroma and the cancer cells might differentiate toward two directions: one was the well-differentiated direction and the other was poorly-differentiated direction. The former was represented by cancer cells showing drug-induced squamous and keratinizing metaplasia. These squamous and keratinizing metaplastic cancer cells formed tumor nests or islands with central keratin pearls (Figs. 2 and 3) and in the extreme condition cancer cells reached the final maturation and

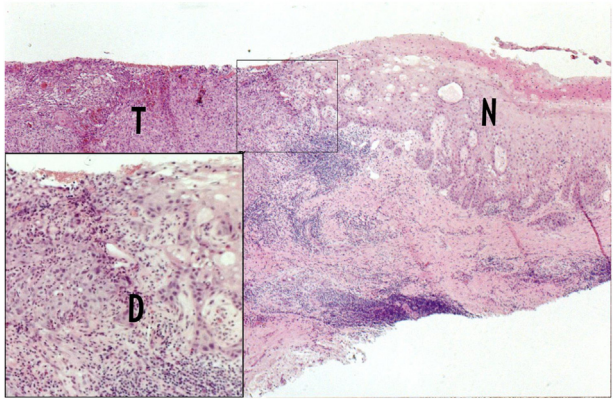

Figure 1 Low-power and high-power microphotographs of an incisional biopsy specimen from an OSCC patient before chemotherapy. The low-power view (right half of the tissue section) showed the transition from normal or dysplastic epithelium (N) to the SCC tumor tissue (T) and there might be a demarcation (D) between tumor tissue (T) and adjacent normal or dysplastic epithelium (N) (H&E stain, original magnification; right microphotograph, 10 × ; inset, 25 ×).

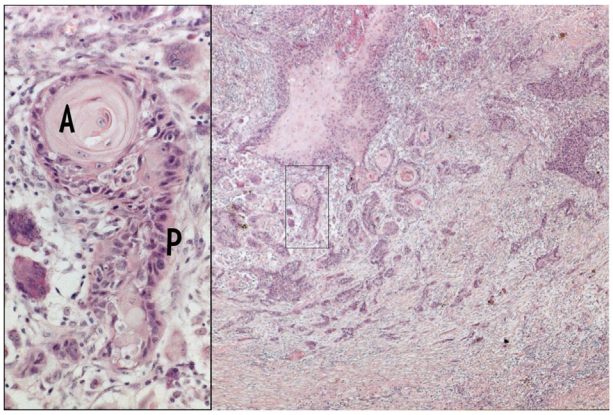

Figure 2 Microphotographs of a surgical excision specimen from an OSCC patient after chemotherapy. Low-power microphotograph (right side) showing small nests of cancer cells invading down into the underlying inflamed fibrous connective tissue stroma. High-power microphotograph (inset) of a drug-induced residual cancer nest composed of the affected (A) and proliferating (P) cancer cells. The affected cancer cells were well-differentiated and keratinized cancer cells, while the proliferating cancer cells were less-differentiated and more pleomorphic and hyperchromatic cancer cells that might be the drug-resistant cancer cells (H&E stain, original magnification; right microphotograph, 10 × ; inset, 25 ×).

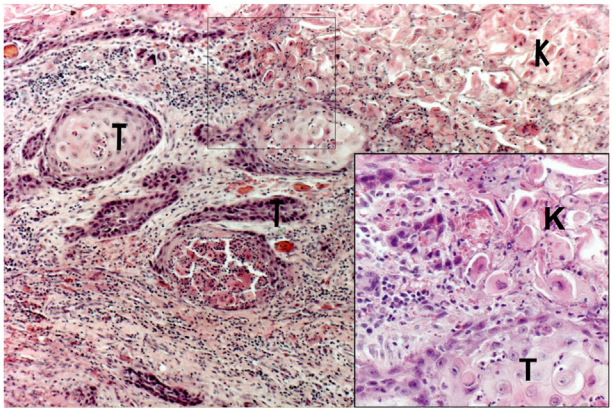

Figure 3 Microphotographs of a surgical excision specimen from an OSCC patient after chemotherapy. Low-power (left side) and high-power (inset) microphotographs showing variation in the extent of differentiation of the residual cancer cells. There were viable cancer cells arranged in nests (T) and squamous and keratinizing metaplastic cancer cells which finally formed individual cell keratinization and keratin pearls (K) (H&E stain, original magnification: left microphotograph, 25 × ; inset, 50 ×).

further transformed into individual keratinized cells or formed keratin pearls (Fig. 3). If the degenerating cancer cell debris, individual keratinized cells, or keratin pearls were directly exposed to the inflamed fibrous connective tissue stroma, macrophages and multinucleated foreign body giant cells might migrate and surround these degenerating and keratinized cancer cells or keratin pearls and executed their phagocytic function (Figs. 4—6).

The cancer cells that went toward the poorly-differentiated direction were drug-resistant cancer cells. They were composed of individual or small aggregates of proliferating cancer cells with pleomorphism, hyperchromatism, high nuclear/cytoplasmic ratio, and mitotic features (Fig. 2). Moreover, these proliferating cancer cells were usually located in the peripheral portion of the primary OSCC lesions and occasionally they were found at the periphery of the affected cancer nests (Fig. 2). In addition, these proliferating cancer cells were usually lack of prominent intercellular bridges, individual cell keratinization, and keratin pearl formation. Therefore, these cancer cells appeared to be less differentiated and more invasive than the cancer cells in the untreated OSCC lesions and the affected cancer cells in the treated OSCC lesions. They were also the main population of the residual cancer cells in the surgical excision specimens of the cases with recurrent OSCC lesions.

Regional cervical lymph nodes were dissected out from the removed soft tissue specimens obtained from upper neck dissection or radical neck dissection. Gross investigation of the lymph nodes dissected out from these neck dissection soft tissue specimens revealed that the lymph nodes with metastatic cancers were usually larger than those without cancer metastasis. However, there were also a few lymph nodes that did not enlarge but contained foci of metastatic cancer cells which were confirmed by subsequent histological examination. For the lymph nodes with obvious metastatic cancer foci, they frequently presented as several solid white areas surrounded by brown nodal tissues at the periphery on the cut surface. The centers of the solid white areas were usually filled with cheesy and muddy materials. These specific features had not been discovered in the metastatic cervical lymph nodes resected from OSCC patients who did not receive adjuvant chemotherapy or irradiation therapy.

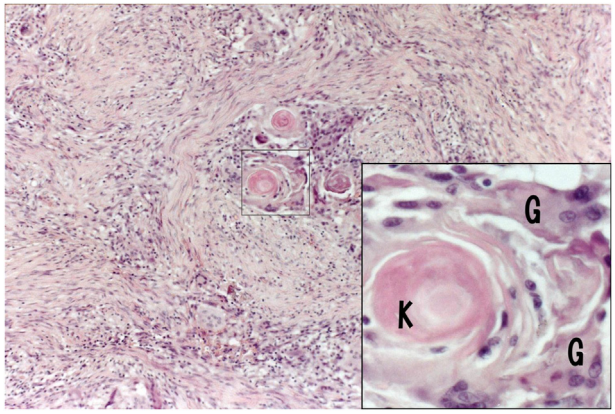

Figure 4 Microphotographs of a surgical excision specimen from an OSCC patient after chemotherapy. Low-power (left side) and high-power (inset) microphotographs showing the inflamed fibrous connective tissue stroma and the residual cancer cells with formation of a keratin pearl (K) which was exposed to the stroma and subsequently surrounded by multinucleated foreign body giant cells (G). (H&E stain, original magnification; left microphotograph, 25 × ; inset, 100 ×).

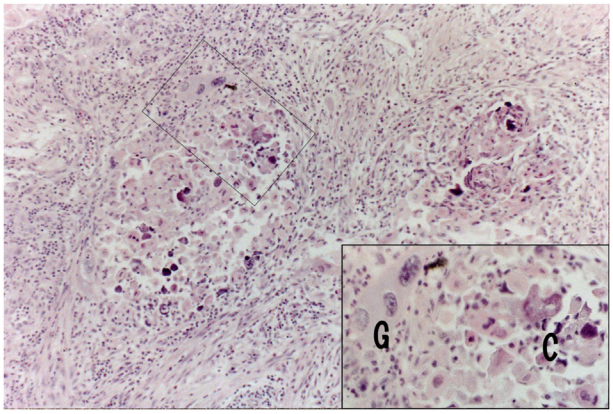

Figure 5 Microphotographs of a surgical excision specimen from an OSCC patient after chemotherapy. Low-power microphotograph (left side) showing the inflamed fibrous connective tissue stroma and the degenerating residual cancer cells with keratinization and calcification. High-power microphotograph (inset) exhibiting some of the keratinized and calcified cancer cells (C) which were surrounded by multinucleated foreign body giant cells (G) (H&E stain, original magnification; left microphotograph, 25 × ; inset, 50 ×).

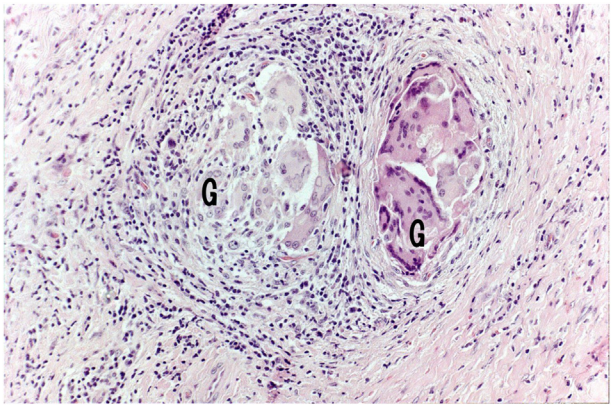

Figure 6 A microphotograph of a surgical excision specimen from an OSCC patient after chemotherapy showing aggregates of the multinucleated foreign body giant cells (G) in the inflamed fibrous connective tissue stroma and phagocytosis of drug-induced and keratinized cancer cell by the multinucleated foreign body giant cells (G) (H&E stain, original magnification, 50 ×).

In the cervical lymph node metastatic cancer lesions after chemotherapy, the residual cancer cells were histologically similar to those seen in primary OSCC lesions after chemotherapy. Therefore, both drug-induced squamous and keratinizing metaplastic cancer cells and drug-resistant proliferating cancer cells could be observed. However, the most characteristic feature in the cervical lymph node metastatic cancer lesions after chemotherapy was the presence of epidermoid cyst-like lesions. These epidermoid cyst-like lesions were lined by a layer of stratified squamous epithelium originating from the drug-induced squamous metaplastic cancer cells and filled with keratin materials in the cystic lumen (Fig. 7). The fibrous cystic wall was constituted with residual lymphoid tissue. Sometimes, cords or strands of cancer cells proliferating from the cystic lining epithelium could also be found in the fibrous cystic wall of an epidermoid cyst-like lesion (Fig. 7). Within these metastatic cervical lymph nodes, lymphocytes were the major inflammatory cells against cancer cells. When the individual keratinized cancer cells or keratin pearls were exposed to the inflamed fibrous connective tissue stroma, they might be phagocytosed by macrophages or multinucleated foreign body giant cells as those seen in the surgical excision specimens of primary OSCCs after chemotherapy (Fig. 8).

When the metastatic lymph node lesions were compared with their primary OSCC lesions after chemotherapy, the cancer cells in the metastatic lymph node lesions showed a more prominent tendency toward increased squamous differentiation and keratinization. Cancer cell keratinization and keratin pearl formation were so prominent that keratin materials became the major constituents of the metastatic lymph nodes. The grayish, cheesy, muddy materials in the cystic cavities of these metastatic lymph nodes was further proved to be keratin materials microscopically (Fig. 7).

Discussion

In this study, despite the wide variations among the OSCC cases studied, several common histological findings were observed in the resected primary OSCC tissues after chemotherapy. These common histological features were summarized as follows. First, residual cancer cells were

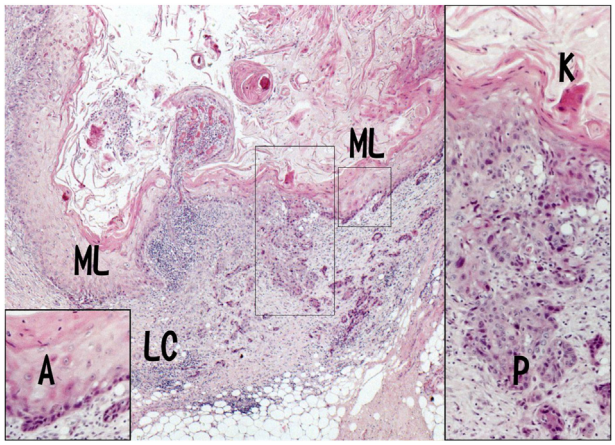

Figure 7 Microphotographs of a cervical lymph node metastatic cancer specimen from an OSCC patient after chemotherapy. Lower-power microphotograph (left side) of a lymph node metastatic cancer lesion (ML) showing formation of an epidermoid cyst-like lesion lined by a layer of stratified squamous epithelium from the affected cancer cells (A, left inset) and production of a lot of keratin materials in the cystic lumen (upper part of the left microphotograph). Some of the proliferating cancer cells (P, right inset) with pleomorphism and hyperchromatism grew down from the stratified squamous lining epithelium toward the "capsule" of the metastatic lymph node (LC) (H&E stain, original magnification; left microphotograph, 10 × ; left or right inset, 25 ×).

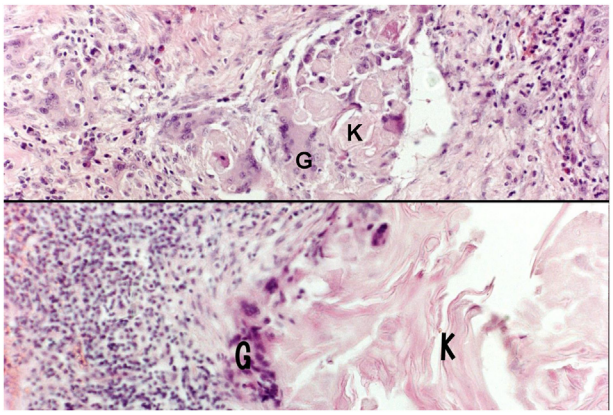

Figure 8 Microphotographs of a cervical lymph node metastatic cancer specimen from an OSCC patient after chemotherapy. Upper microphotograph showing phagocytosis of keratinized cancer cells (K) by multinucleated foreign body giant cells (G). Lower microphotograph demonstrating phagocytosis of drug-affected cancer cells-produced keratin materials (K) by multinucleated foreign body giant cells (G) in the severely inflamed connective tissue stroma of a cervical metastatic lymph node. (H&E stain, original magnification; upper or lower microphotograph, 50 ×).

consistently present. Compared to the incisional biopsy specimen of untreated primary OSCC, residual cancer cells showed an extraordinary tendency toward squamous and keratinizing metaplasia with individual cell keratinization and production of a great amount of keratin pearls. Second, necrosis and degeneration of individual cancer cells were observed in those cases operated soon after the cessation of chemotherapy. Third, the fibrous connective tissue stroma adjacent to the residual cancer cells exhibited variable extents of fibrosis and inflammation. Fourth, the stratified squamous epithelium adjacent to the primary OSCC tissues showed epithelial atrophy or surface ulceration. These findings were similar to those found in a previous study.[9] Moreover, they observed that the extent of cancer cell necrosis and degeneration decreases significantly in OSCC patients who receive surgery between 3 days and 2 weeks after chemotherapy. Cancer cell differentiation and keratinization increased in primary OSCC tissues resected later than 2 weeks after chemotherapy. Furthermore, the tendency toward keratinization became more prominent as time went by. The affected cancer cells exerted squamous and keratinizing metaplasia, but the proliferating cancer cells still grew rapidly. If the proliferating cancer cells were not excised and left in patients, they would finally develop into recurrent OSCC lesions. In this study, the resected specimens of the recurrent OSCC lesions which had been treated with chemotherapy were mainly composed of actively proliferating cancer cells. This finding indicates that OSCC cells were selected by chemotherapeutic agents and these residual proliferating cancer cells may be drug-resistant cancer cells. It is well known that the gain of surgical resectability is important for OSCC patient receiving chemotherapy. However, to avoid the emergence of proliferating cancer cells, a radical operation should be performed soon after the patients recovered from the immunosuppressive effects of the cytotoxic therapeutic agents.[9]

In experimental studies using either tissue cultures or animal models, cytotoxic therapeutic drugs for OSCC cause death of cancer cells within 1—4 days after application.[10,11] In human clinical studies, increased keratinization of affected cancer cells is the common histological finding, but death of cancer cells has rarely been mentioned.[10,12,13] Based on the results of this study, we could support the sequential events occurring in OSCC treated by chemotherapy reported in a previous study.[9] The OSCC cells evolve through 2 stages in response to chemotherapy, an early phase of necrosis and degeneration of cancer cells, and a late phase of squamous and keratinizing metaplasia of cancer cells. The early phase of cancer cell change occurs mainly in the first 4 days and lasts up to 2 weeks after chemotherapy. Afterwards, the drug-induced residual cancer cells undergo a progressive change toward increased keratinization. This late phase of keratinizing change may continue, if time allowed, until most of the cancer cells reach a final suicide status of individual cell keratinization and formation of keratin pearls.[10,12] Thus, we might interpret and establish the mechanism of these two events. In the early phase, the "tumorocidal" effects of chemotherapeutic agents caused death of cancer cells directly. In the late phase, the "tumorostatic" effects of chemotherapeutic agents held the remaining cancer cells in the G_0 phase, but these residual cancer cells finally went to the end of their life as keratinized cells.

It is self-explained that death of cancer cells in the early phase causes immediate shrinkage of the tumor volume.[9] Moreover, the final squamous and keratinizing metaplasia of cancer cells in the late phase results in further shrinkage of tumor volume after chemotherapy, because keratinization of cancer cells involves a process of dehydration, loss of cell organelles, and production of keratin, and these sequential events all lead to a significant reduction of individual cell volume.[9,14] Besides, phagocytosis of the cancer cell debris and keratin materials by macrophages and multinucleated foreign body giant cells as well as the stromal fibrosis also contribute to the reduction of tumor volume.[9] It is by these mechanisms that remarkable or even complete clinical regression of tumor volume has been encouragingly observed in the cases of OSCC treated by adjuvant chemotherapy as seen in other case series of studies.[9,15—17]

On the other hand, although the lymph node metastatic cancer lesions showed more advanced extent of keratinization than their primary OSCC lesions, they usually enlarged after chemotherapy. In these enlarged metastatic lymph nodes, the cancer cell volume really reduced after chemotherapy, but the necrotic cancer cell debris and keratin materials produced by the drug-induced squamous and keratinizing metaplastic cancer cells could not be resorbed by scavenger cells (macrophages and multinucleated foreign body giant cells) and were subsequently accumulated in the central portions of the metastatic lymph nodes to become the contents of the "epidermoid cysts". This can explain why the lymph node metastatic cancer lesions enlarge, despite the fact that

metastatic cancer cells still undergo a prominent process of keratinization.

After chemotherapy, the residual proliferating cancer cells were frequently observed in different proportions in patients. These proliferating cancer cells were less differentiated and more invasive than the original cancer cells and drug-affected squamous and keratinizing metaplastic cancer cells. Therefore, these proliferating cancer cells seemed to be resistant to the cytotoxic chemotherapeutic agents.[13,18] Actually, the presence of drug-resistant cancer cells has been proven in vitro.[19] In addition, cystic change of the lymph node metastatic cancer lesions has been recognized in untreated or radiation-treated patients for many years, especially in carcinomas of the head and neck.[20,21] Lymph node metastatic cancer lesions that showed more advanced extent of keratinization than their primary cancer lesions did have been observed in patients with esophageal SCC.[22] In this study, the lymph node metastatic cancer lesions without foreign body reaction after adjuvant chemotherapy consisted of cancer cell debris and keratin materials accumulated in the lumen of a cyst-like lesion lined by a layer of stratified squamous epithelium, giving the metastatic lymph node looking like a cystic lesion. Although this metastatic lesion appears like a cyst, it should not be mistaken for an epidermoid cyst.

However, residual cancer cells were consistently present in all surgical excision specimens after chemotherapy in this study. In many cases, the residual cancer cells including both the affected and proliferating cancer cells formed small cords, strands, or nests in the inflamed fibrous connective tissue stroma covered by the intact oral mucosal epithelium. Unfortunately, such dispersed residual cancer cell nests might escape from clinical detection and subsequent surgical resection. After chemotherapy, if the drug-treated OSCC is resected according to a "contracted tumor volume", some of these dispersed residual cancer cell nests might be left in the patient.[9] This may cause the development of recurrent OSCC lesions and raise the risk of cancer metastasis. If the recurrent OSCCs are from the drug-resistant proliferating cancer cells, they may have a strongly destructive effect on the local oral tissues of the patient. Therefore, to avoid rapid development of the recurrent OSCC lesions from the residual proliferating cancer cells, surgical resection of the primary OSCC lesions should be performed as soon as the patients' conditions permit after chemotherapy. Meanwhile, a less radical surgery after chemotherapy should be done carefully to avoid the incomplete removal of the residual cancer cells.[23] If possible, it is safer to perform a wide resection of drug-treated OSCC even if the cancer shows a marked regression after chemotherapy.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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