Extraosseous osteogenic sarcomas (EOSs) occur rarely, accounting for 1% of all soft tissue sarcomas and 4% of all osteogenic sarcomas. EOSs are biologically highly malignant neoplasm with an average 5-year survival rate of 15.6%. The tumors usually present in patients over age 40, in contrast with the juvenile predilection for intraosseous osteogenic sarcomas. The common anatomic sites of EOS are the lower extremities, thigh, and buttocks. The head and neck are unusual sites of EOS, only six cases being reported in the literature. Apart from an uninformed case, four of the cases were primary lesions, the remaining case being associated with radiotherapy. We here attempt to determine the pathogenesis in a patient of an unusual EOS lesion that developed in the soft tissue of the neck after ablative surgery and radiotherapy for oral squamous cell carcinoma (SCC).

II. Case report

A 66-year-old male initially presented with an unhealed extraction socket of three months duration. A series of examinations revealed gingival cancer with mandibular invasion. The patient underwent wide excision including segmental mandibulectomy with simultaneous functional neck dissection and reconstruction procedures with a pectoris major myocutaneous flap and reconstruction plates. Microscopically, the tumor was diagnosed as invasive squamous cell carcinoma arising from the gingiva with a metastatic lymph node (pT4N1M0). The mass produced adequate amounts of keratin, being thus regarded as moderately differentiated. We here attempt to determine the pathogenesis in a patient of an unusual EOS lesion that developed in the soft tissue of the neck after ablative surgery and radiotherapy for oral squamous cell carcinoma (SCC).

Key words: Squamous cell carcinoma (SCC), Extraosseous osteogenic sarcoma (EOS), Epithelial-mesenchymal transition (EMT), Snail, E-cadherin, Mouth neoplasms

Abstract

Postirradiation extraosseous osteogenic sarcomas are uncommon in the head and neck, despite the extensive use of high-dose radiation. It has been described as de novo radiation-induced neoplasm. We present a 73-year-old male who had been treated by radiotherapy for gingival cancer 7 years earlier and later developed extraosseous osteogenic sarcomas (EOSs) of the neck. Microscopically, the neck mass was composed with mesenchymal malignant cells with cartilaginous and osteogenic differentiation. Immunohistochemical stain demonstrated strong positivity of tumor cells for Snail, the one of major epithelial-mesenchymal transition (EMT) inducer. The E-cadherin expression was scarce, showing inverse relationship to Snail expression. Compared with previous squamous cell carcinoma (SCC) of the gingiva, the present EOS sample revealed the remained epithelial cells on cytokeratin immunohistochemistry, suggesting the tumor arise from the cells of epithelial origin. We have also reviewed the previous 6 cases of head and neck EOSs carefully. The clinicopathologic features of the unusual lesion suggest that it is an incomplete EMT of precedent epithelial malignancy rather than de novo pathology.

Key words: Squamous cell carcinoma (SCC), Extraosseous osteogenic sarcoma (EOS), Epithelial-mesenchymal transition (EMT), Snail, E-cadherin, Mouth neoplasms
Snail antiserum was prepared as previously described. Polyclonal anti-cytokeratin, Snail, E-cadherin, and N-cadherin. Anti-cytokeratin, anti-E1/3 (Dako, Carpinteria, USA), anti-E-cadherin (Invitrogen, Carlsbad, USA), and anti-N-cadherin (Invitrogen, Carlsbad, USA) were purchased. Polyclonal anti-cytokeratin, Snail, E-cadherin, and N-cadherin. Anti-cytokeratin, anti-E1/3 (Dako, Carpinteria, USA), anti-E-cadherin (Invitrogen, Carlsbad, USA), and anti-N-cadherin (Invitrogen, South San Francisco, USA) were purchased. Polyclonal anti-Snail antisera was prepared as previously described. The SCC samples showed diffuse marked cyttoplasmic cytokeratin expression and nuclear Snail expression in invasive front area. The expression of membranous E-cadherin was scarce, but remained. The N-cadherin expression could not be found. In EOS sample, the presence of a tiny portion of epithelial tumor cells was proved by cytokeratin immunohistochemical staining. Snail was strongly expressed in the nucleus of entire mesenchymal tumor cells, but E-cadherin expression could not be found. Some mesenchymal tumor cells showed membranous positivity for anti-N-cadherin, which was inverse relation to E-cadherin expression.

Analyses of head and neck EOSs This is the seventh known case of head and neck EOS. Previously known anatomic sites of head and neck EOS include lip, chin, zygoma area, and parotid gland. Excluded from these data was a 10-year-old who developed osteogenic sarcoma in the soft tissue of the right orbit following radiotherapy for retinoblastoma, later suggested to be of intraosseous origin.

Interestingly, the third case in Table 1 shares features with ours in its clinical course: the existence of SCC at primary sites, prolonged latency after radiotherapy, and development of EOS in a previously irradiated region. The biologic mechanism causing such unique clinicopathologic features remains obscure. It is unclear whether there are links between either radiotherapy or primary SCC and successive EOS lesions.

The EOS could occur de novo, and some of them resemble postirradiation sarcoma, a rare complication of radiotherapy. Radiation induces sarcoma in 0.2% of patients who have undergone radiotherapy for over 5 years. The chin lesion in the third case might have developed from non-neoplastic myositis ossificans into osteogenic sarcoma following repeated irradiation, and could thus be regarded as radiation-induced EOS. However, the relationship between EOS and previous carcinoma of multiple skin lesions remain to be explained.

The EOS could alternatively result from conversion into mesenchymal tumor of pre-existing epithelial malignancy. This might account for both cases in Table 2. The EOS lesions occurred where epithelial cancer cells had previously presented, possible instances of irreversible transition to mesenchymal tumor of recurrent or metastatic SCC.

Discussion The EMT is a complex program whereby epithelial cells lose their polarity and cell-cell contacts and undergo a dramatic remodeling of the cytoskeleton with acquisition of mesenchymal expression components and manifestation of a migratory phenotype. Down-regulation of E-cadherin function is well known to mark the initiation of the EMT. Snail family can act
Fig. 1. A. Microscopically, primary gingiva lesion showed cords and nests of epithelial cells exhibiting aberrant accumulation of keratin. (arrow)  B. Second primary tumor of buccal mucosa showed severe cellular abnormalities of hyperchromatism and pleomorphism. Scant production of keratin is notified. C–D. Microscopic feature revealed that lesion of the neck was composed of sarcomatous spindle cells with hypercellular cartilage and irregular osteoid. (D, asterisk) (H&E staining, original magnification A, B, and D: x200, C: x100) (SCC: squamous cell carcinoma, EOS: extraosseous osteogenic sarcoma)

Fig. 2. Axial CT scan reveals a neck mass (asterisk) of approximately 3.0×3.0 cm in left level III. (CT: computed tomography)

Fig. 3. A. Immunohistochemical stain for previous SCC showed intense cytoplasmic staining for cytokeratin AE1/3, whole epithelial cell marker. B. Snail was detected in the nucleus of peripheral carcinoma cells. C. The cytoplasmic membranous expression of E-cadherin was occasional. D. The expression of N-cadherin could not be identified. E. The present EOS revealed the minimum positivity for cytokeratin Immunohistochemical staining. F. Sarcoma cells surrounding and within abnormal cartilage (asterisk) showed definitely strong Snail expression. G. E-cadherin disappeared through whole tumor samples. H. The scarce cytoplasmic membranous expression of N-cadherin was detected among the mesenchymal tumor cells. (Immunohistochemical stain, A and E: cytokeratin AE1/3, B and F: Snail, C and G: E-cadherin, D and H: N-cadherin, original magnification x200) (SCC: squamous cell carcinoma, EOS: extraosseous osteogenic sarcoma)
Epithelial-mesenchymal transition in osteogenic sarcoma of the neck following oral squamous cell carcinoma

During the cancer cell invasion and developmental cell migration, the down-regulation of E-cadherin and up-regulation of N-cadherin simultaneously. (E-to-N switch)

Although it is also well known that epithelial cancer cells can be reversibly or irreversibly converted into mesenchymal cells in vitro, EMT was until recently not recognized as a distinct process for cancer progression because 1) it cannot be traced in human tumor samples and 2) carcinomas and sarcomas are thought to interconvert. Recently, pathological activation of the major EMT inducer, Snail, has been reported in a variety of primary tumors. In epithelial tumors, Snail protein expression is restricted to dedifferentiated mesenchyme-like cancer cells and stromal cells placed at the invasive tumor front. In our second primary SCC specimen samples, nuclear Snail expression was also noted in peripheral invasive front zone, in which there was no E-cadherin expression.

EMT may be a transient process that affects only a small fraction of the tumor cell population at any given time. The so-called “sarcomatoid carcinomas” represent a static feature of incomplete EMT with a mixed carcinoma/sarcoma appearance. The majority of tumor cells in the present case showed mesenchymal phenotype and definitely strong Snail expression, with a few showing epithelial characteristics in immunohistochemical staining. These also reflect an incomplete EMT, on the basis of histopathological features.

The patient in the first case also had EOS of lower lip with precedence of SCC in the same region. The interval between the different tumors was more than 3 years, and the lesion was, interestingly, unrelated to radiotherapy. These findings suggest that EMT could actually occur in vivo.

Metastatic carcinoma cells often show a redifferentiated epithelial phenotype. These results suggest that malignant progression, such as invasion and metastasis of cancer cells, might stem from a dynamic and transient EMT program, possibly regulated by epithelial-mesenchymal interactions in the microenvironment. As the two EOS lesions in Table 2 occurred in a previous irradiated region, it may be that radiation affects the tumor microenvironment and regulates EMT of pre-existing carcinoma cells.

---

Table 1. Clinicopathologic data of EOS in the head and neck

<table>
<thead>
<tr>
<th>Case</th>
<th>Author, year</th>
<th>Gender, age</th>
<th>Location</th>
<th>History of RTx</th>
<th>Treatment</th>
<th>Recur/Mets</th>
<th>Status</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Parsons, 1944</td>
<td>M, 53</td>
<td>Lip</td>
<td>No</td>
<td>Excision</td>
<td>Local recur,</td>
<td>Die 24 hrs</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mandible mets</td>
<td>after operation</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Juassawalla, 1964</td>
<td>M, 48</td>
<td>Submental</td>
<td>No</td>
<td>Excision</td>
<td>Probable cerebral mets</td>
<td>Dead</td>
<td>9 mos</td>
</tr>
<tr>
<td>3</td>
<td>Shanoff, 1967</td>
<td>M, 48</td>
<td>Chin</td>
<td>Yes</td>
<td>Excision</td>
<td>Skull mets</td>
<td>Dead</td>
<td>2 1/2 yrs</td>
</tr>
<tr>
<td>4</td>
<td>Das Gupta, 1968</td>
<td>M, 41</td>
<td>Rt. Zygoma</td>
<td>No</td>
<td>Excision</td>
<td>No</td>
<td>Alive</td>
<td>12 1/2 yrs</td>
</tr>
<tr>
<td>5</td>
<td>Sordillo, ?</td>
<td>No info</td>
<td>Face</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Present case</td>
<td>M, 66</td>
<td>Neck</td>
<td>Yes</td>
<td>Excision</td>
<td>No</td>
<td>Alive</td>
<td>5 mos</td>
</tr>
</tbody>
</table>

1 The first lip lesion was SCC, but the recurred tongue was osteogenic sarcoma. (more than 3 years) The metastatic mandibular lesion (within 7 months) was diagnosed radiologically, and necropsy was not permitted.

2 There is no related information.

(SCC: Squamous cell carcinoma, EOS: extraosseous osteogenic sarcoma)

Table 2. EOS associated with radiotherapy in the head and neck

<table>
<thead>
<tr>
<th>Case</th>
<th>Site and reason for RTx</th>
<th>Previous treatment</th>
<th>Amount of radiation</th>
<th>Interval to development of EOS</th>
<th>Site of EOS</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Head and neck, chest and arm</td>
<td>Excision, electrodesiccation, and irradiation</td>
<td>unknown</td>
<td>14 yrs</td>
<td>Chin</td>
<td>History of benign lesion¹</td>
</tr>
<tr>
<td>7</td>
<td>Gingiva and neck for SCC of gingiva</td>
<td>Segmental mandibullectomy and cervical neck node dissection</td>
<td>9,900 cGy</td>
<td>7 yrs and 9 mos</td>
<td>Neck</td>
<td>Present case</td>
</tr>
</tbody>
</table>

¹The previous three biopsies showed myositis ossificans.

(SCC: Squamous cell carcinoma, EOS: extraosseous osteogenic sarcoma)
Though there is no reliable informed data on the prognosis of EOS following radiotherapy, it would likely be poorer than that for common recurrent head and neck cancer. Various treatment modalities should be considered with the patient, including resection followed by adjunct chemotherapy.

Better understanding of the mechanisms of carcinogenesis and EMT during cancer progression may clarify these exceptional in vivo phenomena, and also lead to the development of new targets for therapeutic intervention in cancer.

References