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Epithelial-mesenchymal transition in osteogenic sarcoma of the neck following oral squamous cell carcinoma

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Abstract (J Korean Assoc Oral Maxillofac Surg 2010;36:172-6)

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

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Ⅰ. Introduction

Extraosseous osteogenic sarcomas (EOSs) occur rarely, accounting for 1% of all soft tissue sarcomas and 4% of all osteogenic sarcomas[1-3]. 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[4]. The head and neck are unusual sites of EOS, only six cases being reported in the literature[3-8]. 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).

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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.(Fig. 1. A)

The patient started radiotherapy 1 month after surgery, receiving the first week a dose of 4,500 cGy for the tumor bed

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and neck, boosted to 5,400 cGy the next one week. No particular findings were observed during the treatment period. Supplementary reconstructive surgery with an iliac bone graft was applied a year later due to a fatigue fracture of the reconstruction plate.

A second primary tumor mass was identified in the ipsilateral buccal mucosa 20 months after the initial surgery and completely excised. The specimen was an invasive squamous cell carcinoma, poorly differentiated.(Fig. 1. B) The postoperative course was uneventful until the left neck mass recurred 7 years and 9 months after the initial surgery.

Physical examination revealed a movable neck mass of about 3.0×3.0 cm with hard consistency. However, primary intraoral lesion associated with neck mass was not detected. A computed tomography scan showed a lowly attenuated mass with rim enhancement at the hyoid bone level of neck level III, bounded by the left sternocleidomastoid muscle posteriorly with left internal jugular vein anteromedially.(Fig. 2) Fine needle aspiration biopsy revealed a positive result for malignancy, and the lesion was diagnosed as a metastatic lymph node with necrotic center clinically originating from the oral cavity. Salvage neck node dissection was performed and the acquired specimen was submitted for histopathological evaluation.

Histologically, tumor mass surrounded by a fibrous capsule was noted, relatively well demarcated from adjacent tissues. The tumor was mainly composed of spindle-shaped cells exhibiting pleomorphism, hyperchromatism, and frequent mitosis, consistent with a malignant mesenchymal tumor. It contained deposits of hypercellular cartilage (Fig. 1. C) and aberrant osteoid.(Fig. 1. D) There was no microscopic evidence that the region of the neck mass was lymph node, and the squamous differentiation noted in the previous oral cancer lesion could not be identified. The final diagnosis was extraosseous osteogenic sarcoma.

The immunohistochemical stains were done for both second primary SCC and EOS specimen samples by epithelial-mesenchymal transition (EMT)-associated antibody including cytokeratin, Snail, E-cadherin, and N-cadherin. Anti-cytokeratin AE1/3 (Dako, Carpinteria, USA), anti-E-cadherin (Invitrogen, Carlsbad, USA), and anti-N-cadherin (Invitrogen, South San Francisco, USA) were purchased. Polyclonal anti-Snail1 antiserum was prepared as previously described[9,10]. The SCC samples showed diffuse marked cytoplasmic cytokeratin expression and nuclear Snail expression in invasive front area.(Figs. 3. A, B) The expression of membranous E-cadherin was scarce, but remained.(Fig. 3. C) The N-cadherin expression could not be found.(Fig. 3. D) In EOS sample, the presence of a tiny portion of epithelial tumor cells was proved by cytokeratin immunohistochemical staining.(Fig. 3. E) Snail was strongly expressed in the nucleus of entire mesenchymal tumor cells (Fig. 3. F), but E-cadherin expression could not be found.(Fig. 3. G) Some mesenchymal tumor cells showed membranous positivity for anti-N-cadherin, which was inverse relation to E-cadherin expression.(Fig. 3. H)

Ⅲ. Analyses of head and neck EOSs

This is the seventh known case of head and neck EOS.(Table 1) Previously known anatomic sites of head and neck EOS include lip, chin, zygoma area, and parotid gland[5]. Excluded from these data was a 10-year-boy who developed osteogenic sarcoma in the soft tissue of the right orbit following radiotherapy for retinoblastoma[4,11,12], later suggested to be of intraosseous origin[13].

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.(Table 2) 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[14]. Radiation induces sarcoma in 0.2% of patients who have undergone radiotherapy for over 5 years[15]. 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.

Ⅳ. 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[16]. Down-regulation of E-cadherin function is well known to mark the initiation of the EMT. Snail family can act

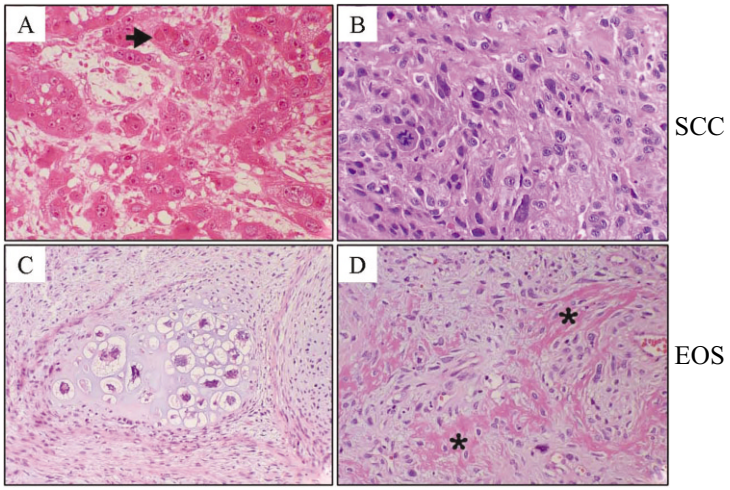

Fig. 1. A. Microscopically, primary gingiva lesion showed cords and nests of epithelial cells exhibiting aberrant accumulation of keratin.(arrow) B. Second primary tumor of buccal mucosa showed severe cellular abnormalities of hyperchromatism and pleomorphism. Scant production of keratin is notified. 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)

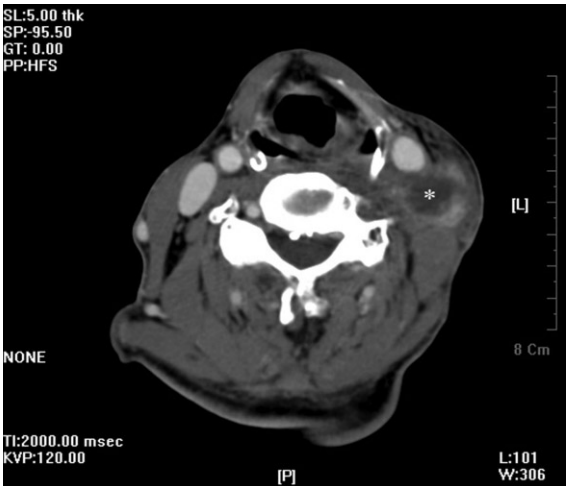

Fig. 2. Axial CT scan reveals a neck mass (asterisk) of approximately 3.0×3.0 cm in left level III. (CT: computed tomography)

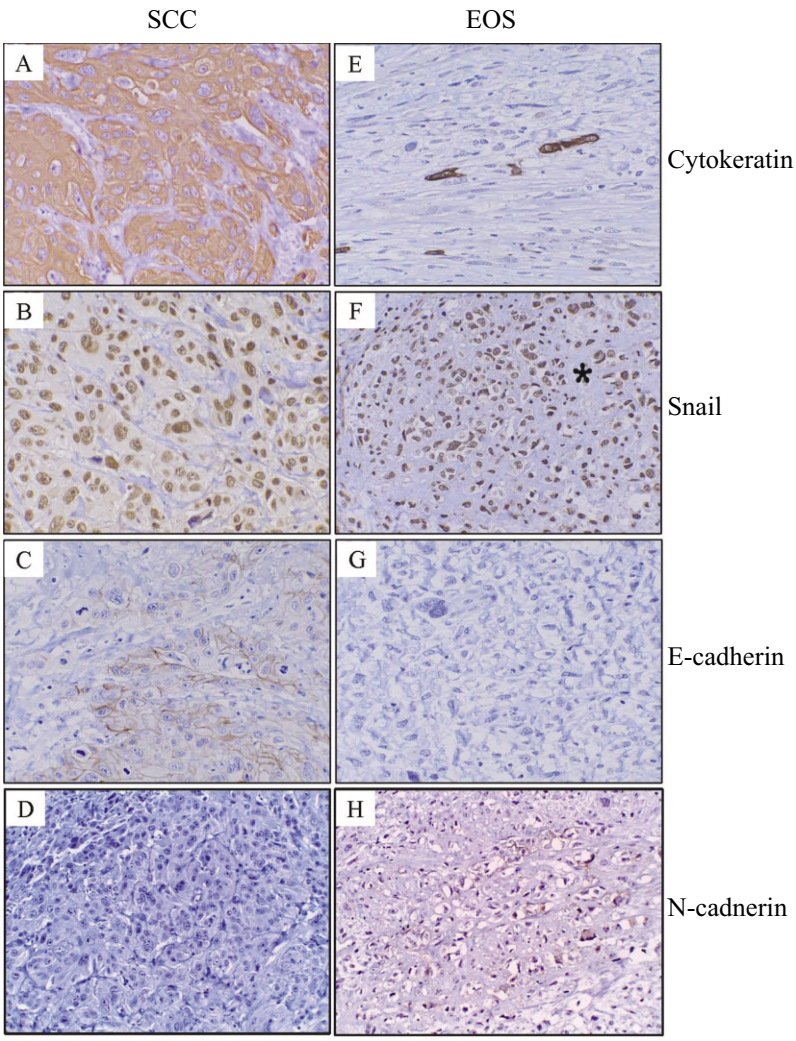

Fig. 3. A. Immunohistochemical stain for previous SCC showed intense cytoplasmic staining for cytokeratin AE1/3, whole epithelial cell marker. B. Snail was detected in the nucleus of peripheral carcinoma cells. C. The cytoplasmic membranous expression of E-cadherin was occasional. D. The expression of N-cadherin could not be identified. E. The present EOS revealed the minimum positivity for cytokeratin Immunohistochemical staining. F. Sarcoma cells surrounding and whinin 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)

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

Case	Author, year	Gender, age	Location	History of RTx	Treatment	Recur/Mets	Status	Follow-up
1	Parsons, 1944	M, 53	Lip	No	Excision	Local recur, Mandible mets[1]	Die 24 hrs after operation	No
2	Juassawalla, 1964	M, 48	Submental	No	Excision	Probable cerebral mets	Dead	9 mos
3	Shanoff, 1967	M, 48	Chin	Yes	Excision	Skull mets	Dead	2 1/2 yrs
4	Das Gupta, 1968	M, 41	Rt. Zygoma	No	Excision	No	Alive	12 1/2 yrs
5	Sordillo, ?[2]	No info	Face	No	-[2]	-[2]	-[2]	-[2]
6	Manning, 1986	M, 73	Rt. Parotid gland	No	Parotidectomy	Unknown	Alive	-[2]
7	Present case	M, 66	Neck	Yes	Excision	No	Alive	5 mos

[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

Case	Site and reason for RTx	Previous treatment	Amount of radiation	Interval to development of EOS	Site of EOS	Remarks
3	Head and neck, chest and arm for multiple skin cancer (SCC) and precancer	Excision, electrodesiccation, and irradiation	unkown	14 yrs	Chin	History of benign lesion[1]
7	Gingiva and neck for SCC of gingiva	Segmental mandibulectomy and cervical neck node dissection	9,900 cGy	7 yrs and 9 mos	Neck	Present case

[1] The previous three biopsies showed myositis ossificans.

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

as transcriptional repressors of E-cadherin and inducers of EMT[17]. 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[18]. Recently, pathological activation of the major EMT inducer, Snail, has been reported in a variety of primary tumors[19]. In epithelial tumors, Snail protein expression is restricted to dedifferentiated mesenchyme-like cancer cells and stromal cells placed at the invasive tumor front[20]. 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[18]. 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[21]. These findings suggest that EMT could actually occur *in vivo*.

Metastatic carcinoma cells often show a redifferentiated epithelial phenotype[22-25]. 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.

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

1. Lin SY, Chen WM, Wu HH, Chen WY, Chen TH. Extraosseous osteogenic sarcoma. J Chin Med Assoc 2005;68:542-5.
2. Rao U, Cheng A, Didolkar MS. Extraosseous osteogenic sarcoma: clinicopathological study of eight cases and review of literature. Cancer 1978;41:1488-96.
3. Sordillo PP, Hajdu SI, Magill GB, Golbey RB. Extraosseous osteogenic sarcoma. A review of 48 patients. Cancer 1983;51:727-34.
4. Manning JT, Raymond AK, Batsakis JG. Extraosseous osteogenic sarcoma of the parotid gland. J Laryngol Otol 1986; 100:239-42.
5. Das Gupta TK, Hajdu SI, Foote FW Jr. Extraosseous osteogenic sarcoma. Ann Surg 1968;168:1011-22.
6. Jussawalla DJ, Desai JG. Primary osteogenic sarcoma arising in extra-skeletal soft tissues of the neck. Br J Surg 1964;51:504-5.
7. Parsons WH, Henthorne JC. Extraskeletal osteogenic sarcoma : report of a case of osteogenic sarcoma of the lip. Ann Surg 1944;119:595-601.
8. Shanoff LB, Spira M, Hardy SB. Myositis ossificans: evolution to osteogenic sarcoma. Report of a histologically verified case. Am J Surg 1967;113:537-41.
9. Yook JI, Li XY, Ota I, Fearon ER, Weiss SJ. Wnt-dependent regulation of the E-cadherin repressor snail. J Biol Chem 2005; 280:11740-8.
10. Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH, *et al*. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nat Cell Biol 2006;8:1398-406.
11. Allan CJ, Soule EH. Osteogenic sarcoma of the somatic soft tissues. Clinicopathologic study of 26 cases and review of literature. Cancer 1971;27:1121-33.
12. Kauffman SL, Stout AP. Extraskeletal osteogenic sarcomas and chondrosarcomas in children. Cancer 1963;16:432-9.
13. Wurlitzer F, Ayala L, Romsdahl M. Extraosseous osteogenic sarcoma. Arch Surg 1972;105:691-5.
14. Kalra S, Grimer RJ, Spooner D, Carter SR, Tillman RM, Abudu A. Radiation-induced sarcomas of bone: factors that affect outcome. J Bone Joint Surg Br 2007;89:808-13.
15. Tan A, Ngan SY, Choong PF. Post-radiation sarcoma of the neck treated with re-irradiation followed by wide excision. World J Surg Oncol 2006;4:69.
16. Oda H, Tsukita S, Takeichi M. Dynamic behavior of the cadherin-based cell-cell adhesion system during Drosophila gastrulation. Dev Biol 1998;203:435-50.
17. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 2007;7:415-28.
18. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442-54.
19. Becker KF, Rosivatz E, Blechschmidt K, Kremmer E, Sarbia M, Höfler H. Analysis of the E-cadherin repressor Snail in primary human cancers. Cells Tissues Organs 2007;185:204-12.
20. Francí C, Takkunen M, Dave N, Alameda F, Gómez S, Rodríguez R, *et al*. Expression of Snail protein in tumor-stroma interface. Oncogene 2006;25:5134-44.
21. Fine G, Stout AP. Osteogenic sarcoma of the extraskeletal soft tissues. Cancer 1956;9:1027-43.
22. Brabletz T, Hlubek F, Spaderna S, Schmalhofer O, Hiendlmeyer E, Jung A, *et al*. Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. Cells Tissues Organs 2005;179: 56-65.
23. Jeschke U, Mylonas I, Kuhn C, Shabani N, Kunert-Keil C, Schindlbeck C, *et al*. Expression of E-cadherin in human ductal breast cancer carcinoma in situ, invasive carcinomas, their lymph node metastases, their distant metastases, carcinomas with recurrence and in recurrence. Anticancer Res 2007;27:1969-74.
24. Liu J, Ikeguchi M, Nakamura S, Kaibara N. Re-expression of the cadherin-catenin complex in lymph nodes with metastasis in advanced gastric cancer: the relationship with patient survival. J Exp Clin Cancer Res 2002;21:65-71.
25. Park D, Kåresen R, Axcrona U, Noren T, Sauer T. Expression pattern of adhesion molecules (E-cadherin, alpha-, beta-, gamma-catenin and claudin-7), their influence on survival in primary breast carcinoma, and their corresponding axillary lymph node metastasis. APMIS 2007;115:52-65.