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Original Article

Epithelial-mesenchymal transition in oral squamous cell carcinoma of the gingiva: An immunohistochemical and ultrastructual case study.

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To know the epithelial-mesenchymal transition in oral squamous cell carcinoma, a case of metastasized squamous cell carcinoma of the gingiva, which represents spindle-shaped cells derived from conventional polygonal/sguamous shaped solid type. We investigated the differences in the expression manner of immunohistochemical and ultrastructural properties between spindle-shaped and polygonal /squamous-shaped carcinoma cell components. Strong immunopositive expression of cytokeratin AE1/AE3, EMA and partially or weak expression of vimentin, CD44 std, CD44 v6, α-catenin and β-catenin were observed. However E-cadherin and CD44 v3 were weak or negative. CD44 v6 showed dynamic their expression, which strongly localized in the cell surface of invading spindle-shaped carcinoma cells and intercellular matrix in the outer layer rather than the center layer in both primary and metastatic tumor cells. These findings suggested that the spindle-shaped carcinoma cells reveal their characteristic configuration based from the acquiring their epithelial-mesenchyamal transition activity differed from the polygonal-shaped carcinoma cells.

Key words: squamous cell carcinoma, epithelial-mesenchymal transition, immunohistochemistry, ultrastructure

Introduction

Spindle-shaped carcinoma cells represented in the squamous cell carcinoma were regarded previously as biphasic tumor cells consisting of both sarcomatoid fusiform proliferation of pleomorphic spindle-shaped cells and squamous-shaped cells, whereas it is nowadays considered as a variant of squamous cell carcinoma. This unusual and controversial type of tumor has been believed to be either a squamous cell carcinoma associated with a typical reactive connective tissue process (pseudosarcoma)[1-4], a collision growth from the combination of a carcinoma and a sarcoma (carcinosarcoma)[5], or a squamous cell carcinoma with spindle cell anaplasia (spindle cell carcinoma)[6, 7]. However, the greater part of the correlation between the morphological change and cellular behavior is not completely cleared[8, 9].

Here we study a case of squamous cell carcinoma showing spindle-shaped morphology and discuss its character in terms of the immunohistochemical and ultrastructural specificities of the carcinoma cells.

Materials and methods

Case study

The patient was a 74-year-old woman, whose chief complaint was gingival swelling of the left molar area. The swelling exhibited a cauliflower-like appearance and reached 35 mm in width and 39 mm in length. Furthermore, swelling was also found to be present in the deep cervical lymph nodes. Serial X-ray examinations, including CT scan, showed the existence of a tumor-like mass and alveolar bone destruction. A biopsy of the gingival tumor was performed and histopathological diagnosis of the squamous cell carcinoma was made. Surgical resection of the carcinoma, including a broad area of alveolar bone, and dissection of the cervical lymph nodes were performed. Histologically, both the biopsy and operated specimens including the lymph nodes revealed an invasive growth pattern of well-differentiated carcinoma cells. The carcinoma consisted of two components: a solid proliferation of atypical squamous cells with dyskeratosis and an invasive proliferation. A peripheral component of the carcinoma was an obvious proliferation of spindle-shaped carcinoma cells. These spindle-shaped carcinoma cells exhibited sheet and strand modes in the same manner as a conventional carcinoma. These carcinoma cells were characterized by elongated cytoplasms and hyperchromatic nuclei. Mitosis was frequently observed among the carcinoma cells (Fig. 1 A and 1 B).

In the deep cervical metastatic lymph nodes (L3 region), the carcinoma exhibited a massive proliferation, and was composed of elements of both polygonal/

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Table 1　Primary antibodies and immunohistochemical results of carcinoma cells in this study.

		Primary	Metastasis
Antibody	Source, M/P*, Dilution	nest / periphery	nest / periphery
Cytokeratin AE1/AE3	DAKO**, M, 1：50	＋／＋	＋／＋
EMA	DAKO†, M, 1：100	＋／－	＋／－
Vimentin	DAKO†, M, 1：50	－／＋	－／－
E-cd	Takara‡, M, 1：100	±／±	±／±
β-ct	Takara‡, P, 1：100	±／±	±／±
α-ct	Takara‡, P, 1：100	±／±	±／±
CD44std	Takara‡, M, 1：50	－／＋	－／＋
CD44v3	R&D, M, 1：10000	－／－	－／－
CD44v6	R&D, M, 1：1000	＋＋／＋＋	＋／＋＋

*monoclonal antibody/ polyclonal antibody; **DAKO CORPORATION, CA, USA; †DAKO A/S, Glostrup, Denmark; ‡Takara biomedicals, ootsu, Japan; R&D Systems, Inc., MN, USA

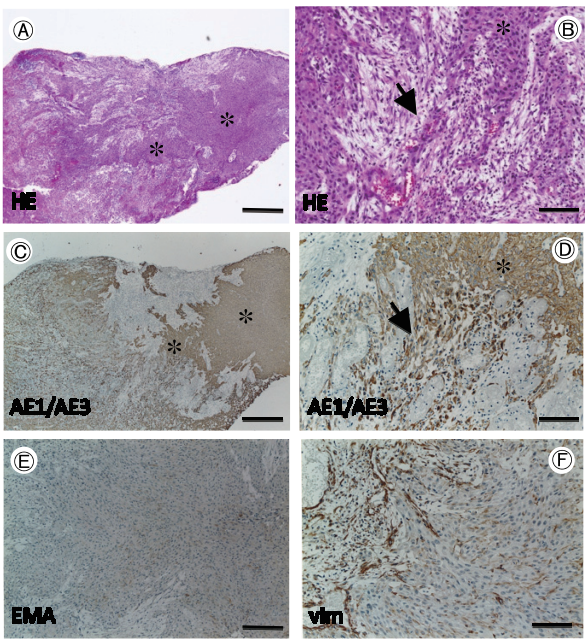

Figure 1 ：Histopathological and immunohistological findings of cellular differentiation in the squamous cell carcinoma of the gingival primary lesion.

Carcinoma composed of two components: solid proliferation of squamous-shaped cells nest（＊）and spindle-shaped peripheral component（arrows）(A and B). The spindle-shaped carcinoma cells exhibited sheet and strand modes with showing cytokeratin（C and D）and EMA（E）expressions. Vimentin showed a slight expression in the spindle-shaped carcinoma cells（F）. HE in A and B; cytokeratin AE1/AE3 in C and D; EMA in E; vimentin in F. Bars＝500μm in A and C；100μm in B, D, E, F

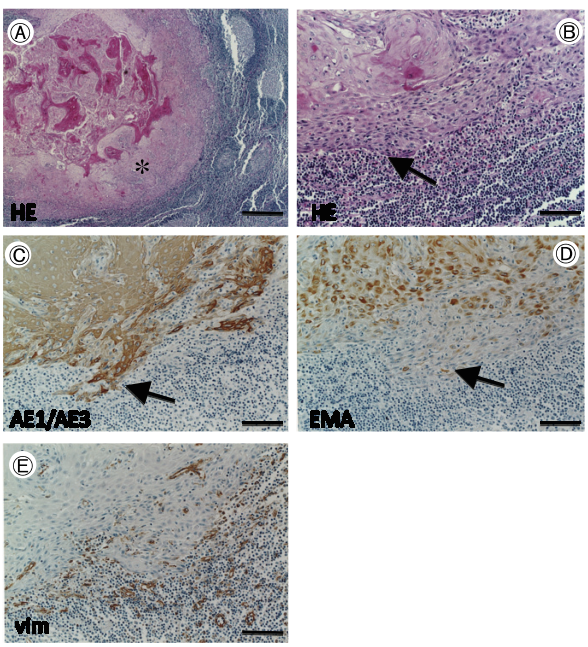

Figure 2 ：Histopathological and immunohistological findings of cellular differentiation in the squamous cell carcinoma of the metastatic cervical lymph node.

In the lymph node metastatic lesion, the carcinoma cells showed a solid proliferation of squamous cell nests（＊）with keratin pearls（A and B）, and the spindle-shaped peripheral components（arrows）with immunopositivity present for cytokeratin（C）but absent of EMA expression（D）. Vimentin showed negative expression in the spindle-shaped carcinoma cells（E）. HE in A and B; cytokeratin AE1/AE3 in C; EMA in D; vimentin in E. Bars＝500μm in A；100μm in B, C, D, E

squamous-shaped and spindle-shaped carcinoma cells. The polygonal/squamous-shaped carcinoma cell nest was located in the center of a carcinoma keratinized mass. However, the peripheral cells developed into spindle shapes (Fig. 2 A and 2 B).

Immunohistological and ultrastructural study

To understand the epithelial-mesenchymal transition in greater detail, paraffin blocks were sliced for histological and immunohistochemical study, or embedded into the epoxy resin（EPON812, TAAB, London, UK）for ultrastructural study using transmission electron microscopy（TEM）. Antibodies used for the present study were listed in table 1, anti-cytokeratin AE1/AE3 and anti-epithelial membrane antigen（EMA）as epithelial cell markers, anti-vimentin as a major mesenchymal cell marker, anti-E-cadherin, anti-α-catenin and anti-β-catenin, and anti-CD44

family series, standard (CD44std), variant 3 (CD44v 3) and variant 6 (CD44v 6) as cellular adhesion molecules, respectively. The immunohistochemistry was performed according to the manufacturer's manual. All clinical data have been allowed by the ethics committee at Asahi University Hospital (#23116).

Results

Immunohistochemical findings

Immunopositive reaction for cytokeratin AE1/AE3 was strong; however EMA was detected in only the center nest of the carcinoma in both the primary lesion and the metastatic lymph nodes. These findings were compatible with the reaction for vimentin observed in the part of the carcinoma cells located only in the primary lesion (Fig. 1 C-F, 2C-E). On the other hand, the reactivity of cell adhesion molecules such as E-cadherin, α-catenin, and β-catenin exhibited a slight positive relationship with the carcinoma cells in both the primary carcinoma cell nest and metastatic lesions (Fig. 3A, 3C, 3E, 4A, 4C, 4E). Immunoreactivity for CD44std was positive at the cell membrane of spindle-shaped cells, but not at polygonal/squamous-shaped cells in either the primary and metastatic lesions. CD44v 3 was not detectable in neither the primaryn or metastatic lesions. Controversially, CD44v 6 exhibited a strong positive reaction at the carcinoma cell surface in both spindle-shaped cells and polygonal/squamous-shaped cells (Fig. 3B, 3D, 3F, 4B, 4D, 4F).

Ultrastructual findings

TEM clarified that the spindle-shaped carcinoma cells in both the primary and metastatic lesions were characterized by the production of a small number of tonofilaments and a desmosome complex as well as polygonal/squamous-shaped cells. The structure of the nucleus in spindle-shaped and polygonal/squamous-shaped cells showed differences in various fields of the carcinoma cells. A number of nucleoli and condensed heterochromatins showed dot-like patches in the spindle-shaped cells, while those of the polygonal/squamous-shaped cells showed slightly distributed (Fig. 5).

Discussion

Histopathology of the present case is characterized by foci of conventional squamous cell carcinoma combined with a transition into a considerable amount of spindle-shaped cell morphology. Such histopathological findings indicate that this malignant tumor must be distinguished both from a squamous cell carcinoma that has provoked a reactive fibroblastic stromal proliferation and from a carcinosarcoma. The spindle-shaped cells in the present case, with various types of atypism, exhibited aggressive behav-

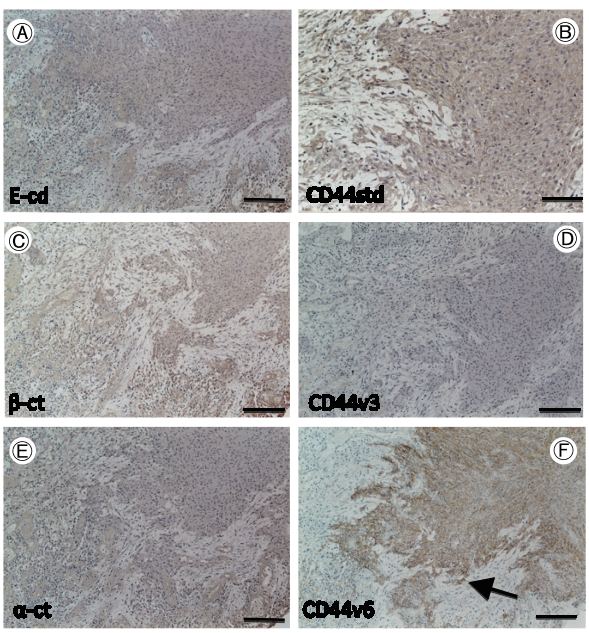

Figure 3 : Immunohistological findings of cellular adhesional molecules in the squamous cell carcinoma of the primary gingival lesion.

E-cadherin (A), β-catenin (C), and α-catenin (E) exhibited slight positive expression in carcinoma cells. Immunoreactivity for the CD44 series: CD44std (B) showed positive expression at the cell membrane in all carcinoma cells, except for CD 44v 3 (D). However, CD44v 6 (arrow) exhibited a strong positive reaction at the carcinoma cell membrane (F). E-cadherin (E-cd) in A; β-catenin (β-ct) in C; α-catenin (α-ct) in E; CD44 std in B; CD44v 3 in D; CD44v 6 in F. Bars = 250μm in A, C, D, E, F ; 100μm in B

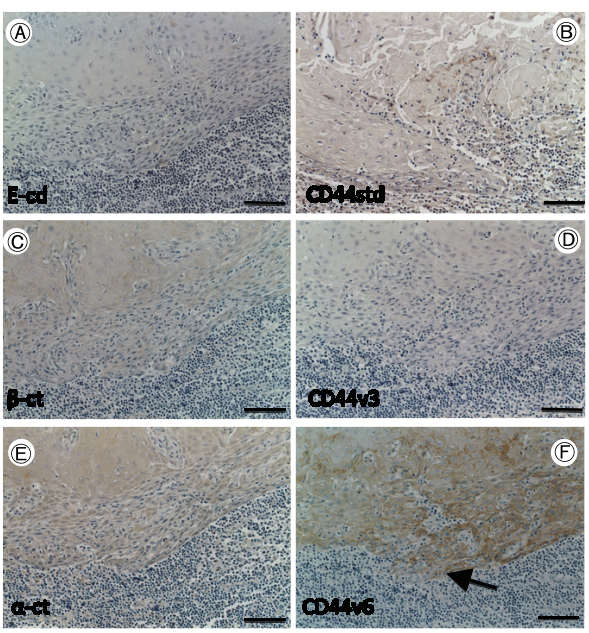

Figure 4 : Immunohistological findings of cellular adhesional molecules in the squamous cell carcinoma of the metastatic lymph node.

E-cadherin (A), β-catenin (C), and α-catenin (E) exhibited slight positive expression in carcinoma cells. Immunoreactivity for the CD44series showed similar positive features, thus CD44std (B) showed positive expression at the cell membrane in all carcinoma cells, except for CD44v 3 (D). However, CD44 v 6 (arrow) exhibited a strong positive reaction at the carcinoma cell membrane. E-cadherin (E-cd) in A; β-catenin (β-ct) in C; α-catenin (α-ct) in E; CD44std in B; CD44v 3 in D; CD44v 6 in F. Bars = 100μm

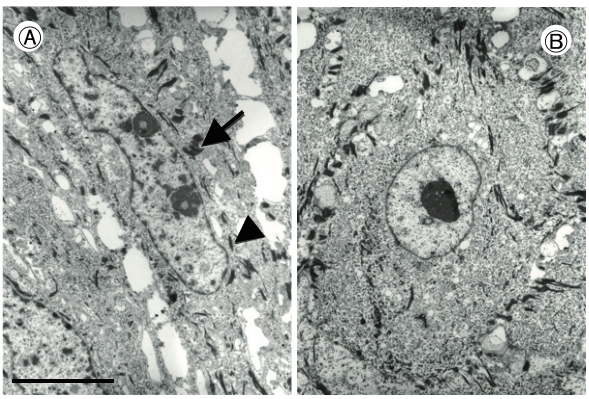

Figure 5 : Ultrastructural findings of carcinoma cells in the spindle-shaped area and polygonal / squamous-shaped area in the primary lesion.

The spindle-shaped carcinoma cells were characterized by a small number of cytoplasmic tonofilaments (arrowhead) and desmosome complex (arrow) with condensed heterochromatins in the nucleus (A), as compared with polygonal / squamous-shaped carcinoma cells (B). Bar = 5 μm

ior, as they induced the bone destruction and metastasis to the lymph nodes, and the spindle-shaped cell proliferation appears to be an unusual cell differentiation. This is due to the fact that it was obvious that the cells are derived from the squamous cell carcinoma through continuing cellular transition. Ultrastructural examination also clarified that the carcinoma cells belong to the epithelial cell because of the production of tonofilaments and a desmosome complex. Furthermore, immunohistochemistry displayed evidence of weak reactivity for vimentin and positive reactivity for cytokeratin AE1/AE3[10]. Common reactivity of cell adhesional molecules, such as E-cadherin and α-catenin, to both tumor cells in this examination, and as reported previously by Navarro[11], suggested that the development of the cell phenotype involves functional disturbance of genes, which regulate cellular differentiation. The difference in reactivity of the CD44 family between polygonal / squamous-shaped and spindle-shaped cells, especially CD44v6 reactivity, indicates that spindle-shaped cells acquire different differentiation to polygonal/squamous-shaped cells. CD44family, the transmembrane glycoprotein is known to have a variety of isoforms due to extensive alternative splicing of 11 of 21 exons of the CD44 gene and various types of post-transcriptional modification[12], and to exhibit a further correlation between increased or decreased expression of variant molecules and metastasis[12–14]. In the foci of carcinoma in this case, CD44std was reactive to spindle-shaped cells, and CD44v3 was negative. CD44v6 was characterized by strong positive to spindle-shaped cells. With regard to the CD44 family in gallbladder carcinomas, it has been reported that CD44std shows a strong reactivity as it is in normal mucosa, but CD44v3 and CD44v6 react to moderately and poorly differentiated areas[14, 15]. There is also evidence that CD44v6 expression is a key for the prognosis in leukemia or malignant lymphomas[16, 17].

From these findings obtained by various examinations and the report according to expression of the CD44 family in carcinoma mentioned above, it was concluded that the unusual spindle-shaped cell type squamous cell carcinoma reveals an abnormal reverse or another process of cellular differentiation with acquisition of epithelial-mesenchyamal transition resulting in the invasive activity of carcinoma cells.

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扁平上皮癌における上皮間葉転換現象とこれに関与する分子：1症例の舌癌における検討

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重層扁平上皮は高度に分化した組織であるが，これを発生由来とする扁平上皮癌ではその分化程度により，本来の組織形態とは異なる形質を発現することが知られている．本研究はこれらの形質発現の変化の中で上皮間葉転換の一つとして知られる扁平上皮から紡錘形の間葉系への分化の可能性とその癌細胞の浸潤性への関連を探るため，超微細構造を含む形態的な変化や細胞間接着因子のカドヘリンとカテニン，細胞外基質のヒアルロン酸と大きく関わり，また腫瘍の浸潤や細胞活性にも関わると言われているCD44ファミリーについて免疫組織化学的に検索した．歯肉に生じた扁平上皮癌で頸部リンパ節転移を示した一例を材料にその検索を行ったところ，原発巣における紡錘形を示す癌細胞は超微細形態的にも紡錘形を示すがトノフィラメントやデスモゾーム構造を有した上皮細胞としての性格を維持しており，また免疫染色においても，ケラチン発現の維持とEMAの減少に加えて間葉成分のビメンチン発現を認めたが，細胞間接着に関する異常は認めなかった．一方，CD44ファミリーのバリアント6（CD44v6）は紡錘形を示す癌細胞に強い発現を示し，この発現と癌細胞の浸潤が強く示唆された．

キーワード：扁平上皮癌，上皮間葉転換，免疫組織化学，超微細構造

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