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Correlation of basic fibroblast growth factor expression with the invasion and the prognosis of oral squamous cell carcinoma

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BACKGROUND: The aim of this study was to evaluate the relationship between the expression of basic fibroblast growth factor (bFGF) and fibroblast growth factor receptor-1 (FGFR-1) in cancer cells and fibroblasts at the invasive front of oral squamous cell carcinoma (OSCC), and the pathologic and clinical characteristics.

METHODS: Sections of 61 biopsy specimens of primary OSCC were immunostained to assess the expression of bFGF and FGFR-1 in cancer cells and fibroblasts at the invasive front.

RESULTS: The bFGF and FGFR-1 expressions in the cancer cells were evident in all specimens, whilst, in fibroblasts, they were detected in 41 (67%) of 61 specimens. These expressions in the fibroblasts occurred notably more often in high-invasive OSCC specimens than low-invasive OSCC specimens. The prevalence of bFGF and FGFR-1 expressions in cases with lymph node metastasis was significantly higher ($P < 0.05$) than in cases without metastasis. Moreover, these expressions were well correlated with patient prognosis.

CONCLUSION: This study concludes that bFGF and FGFR-1 expressions in fibroblasts at the invasive front are linked to the mode of invasion and the prognosis in OSCC.

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Keywords: basic fibroblast growth factor; fibroblasts; invasion; oral squamous cell carcinoma; prognosis

Introduction

The interactions of cancer cells with host stroma play an important role in the progress of solid cancers (1). In particular, interaction(s) between cancer cells and fibroblasts around the tumor, through the mediation of cytokines produced by cancer cells and/or fibroblasts, is considered to be involved in cancer invasion (2, 3).

Basic fibroblast growth factor (bFGF) is a cytokine closely associated with the activation of fibroblasts (4), and can be produced by many cell types (5). It has been demonstrated that the occurrence of bFGF in cancer is higher than that in normal tissues (6). From these bases, the hypothesis can be investigated whether cancer cells and fibroblasts produce bFGF in oral squamous cell carcinoma (OSCC), and if their bFGF production is linked to the characteristics of the cancer.

The aim of this study was to investigate immunohistochemically the presence or absence of bFGF expression in cancer cells and fibroblasts at the invasive front, and the relationship between the bFGF expression and the pathologic and clinical characteristics of OSCC. We also assessed the expression of fibroblast growth factor receptor-1 (FGFR-1), which is regarded as a high-affinity receptor for bFGF (7), in OSCC.

Materials and methods

Patients

Pre-treatment biopsy specimens obtained from 61 patients with primary OSCC (31 males and 30 females; mean age 69 years), treated in our clinic between June 1988 and October 2003, were examined. The origin of cancer was as follows: tongue ($n = 25$), gingiva ($n = 25$), oral floor ($n = 5$), buccal mucosa ($n = 3$), soft palate ($n = 2$), and lips ($n = 1$).

The pathologic futures (differential type and status of invasion) of each specimen were assessed by histologic examination with hematoxylin and eosin (H & E) stain. The grade of tumor differentiation was determined according to the criteria proposed by the World Health Organization. In this study, the invasion status of the tumor was evaluated using criteria by Yamamoto et al. (8; Table 1).

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Table 1 Histologic grading of mode of cancer invasion

Grade	*Histologic character*
1	Well-defined borderline
2	Cords, less marked borderline
3	Groups of cells, no distinct borderline
4C	Diffuse invasion, cord-like type
4D	Diffuse invasion, widespread type

This classification is presented by Yamamoto et al. (8).

The clinical information of each patient, such as the presence or absence of regional lymph node metastasis at biopsy and the prognosis, was obtained from the patient's medical records.

Staining
Each specimen was fixed in 10% buffered formalin, and then embedded in paraffin to prepare serial sections (4 μm). After treating with trypsin solution for 30 min at 37°C, immunohistochemical staining was performed by Labeled Strept Avidin-Biotin method following deparaffinization and rehydration. Endogenous peroxidase was blocked using 0.3% hydrogen peroxidase in PBS for 30 min. The sections were washed with PBS and incubated with casein in phosphate-buffered saline (PBS) for 10 min to block non-specific reactions. As a primary antibody, rabbit polyclonal anti-bFGF antibody (SC-79) or anti-FGFR-1 antibody (SC-121; ×100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was reacted at 4°C for 24 h. The slides were incubated with biotinylated antirabbit immunoglobulin (DAKO, Kyoto, Japan), and reacted at room temperature for 60 min. After reacting with peroxidase-conjugated streptoavidin (DAKO) for 60 min, they were washed with PBS. Immunohistochemical reactions were developed in 3,3′-diaminobenzidine tetrahydrochloride (Wako, Osaka, Japan), then counterstained with hematoxylin. Negative controls were treated with all reagents except the primary antibody.

Criteria for bFGF and FGFR-1 expressions
The expression of bFGF and FGFR-1 in cancer cells and fibroblasts at the invasive front was examined under a microscope with 200× magnification. Positive expression was identified if the cells completely or partially expressed bFGF. The expression of FGFR-1 was assessed in the same manner.

Statistical analysis
Spearman's correlation coefficient by rank test was employed to analyze statistically the relationship between bFGF and FGFR-1 expressions and differential type and the mode of invasion. The chi-square test was used for statistical comparison of the prevalence of these expressions between the presence and absence of lymph node metastasis. Survival rates of bFGF-positive and -negative patients were calculated by the Kaplan–Mayer method, and examined for statistical significance using the log-rank test.

Results

bFGF and FGFR-1 expressions in cancer cells and fibroblasts
Both bFGF and FGFR-1 expressions in cancer cells were evident in all 61 specimens. In contrast, 41 (67%) of 61 specimens were positive for bFGF expression in the fibroblasts. Amongst the 41 bFGF-positive specimens, 34 (83%) were positive for FGFR-1 expression. Neither bFGF nor FGFR-1 was expressed in the fibroblasts of 17 specimens (Fig. 1).

Relationship between bFGF/FGFR-1 expression in fibroblasts and the clinical characteristics of OSSC
Most moderately and poorly differentiated OSCC specimens revealed a positive expression of bFGF and FGFR-1 in the fibroblasts, whilst these expressions were found in 59% of well-differentiated SCC specimens (Table 2). Moreover, these expressions were found to be correlated with the mode of invasion (Table 3). Although the majority of grades 1 and 2 specimens were negative for bFGF and FGFR-1 expressions in the fibroblasts, a high prevalence of these expressions was evident for high invasion grade specimens (grades 3, 4C and 4D). In particular, these expressions in the fibroblasts were evident in all seven 4D specimens.

Eighteen (49%) of 37 cases, where no lymph node metastasis was detected, were positive for bFGF expression in the fibroblasts (Table 4). In contrast, bFGF was expressed in 96% of cases with metastasis. This difference was significant ($P < 0.05$). Similar findings were evident with FGFR-1 expression.

The prevalence of bFGF expression in the fibroblasts of patients with good prognosis was significantly smaller ($P < 0.01$) than in patients with poor prognosis (Fig. 2).

Discussion

We confirmed that the intensity of bFGF and FGFR-1 expressions in cancer cells at non-invasive areas was very low (data not shown). In contrast, these expressions in cancer cells were evident in all specimens. In addition, their intensity was strong in almost all specimens. These results indicate that cancer cells do produce bFGF in all OSCC regardless of the pathologic and clinical characteristics of OSCC. The role of bFGF concerning the progress of cancer has been investigated. The proliferation activity of tumors is correlated with the expression of bFGF in OSCC (9). It has been demonstrated that bFGF promotes the production of cancer cell proteinases and enhances their invasive ability (10). It is reported that bFGF affects fibroblasts on the production of hepatocyte growth factor, transforming growth factor-β, and matrix metalloproteinase-2, which are relevant to tumor invasion (11). Moreover, bFGF exerts pro-angiogenic activity by interacting with various endothelial cell surface receptors (12). On the basis of the results of this study and the reported findings, it is likely that bFGF produced by cancer cells at the invasion front activates the cancer cells themselves

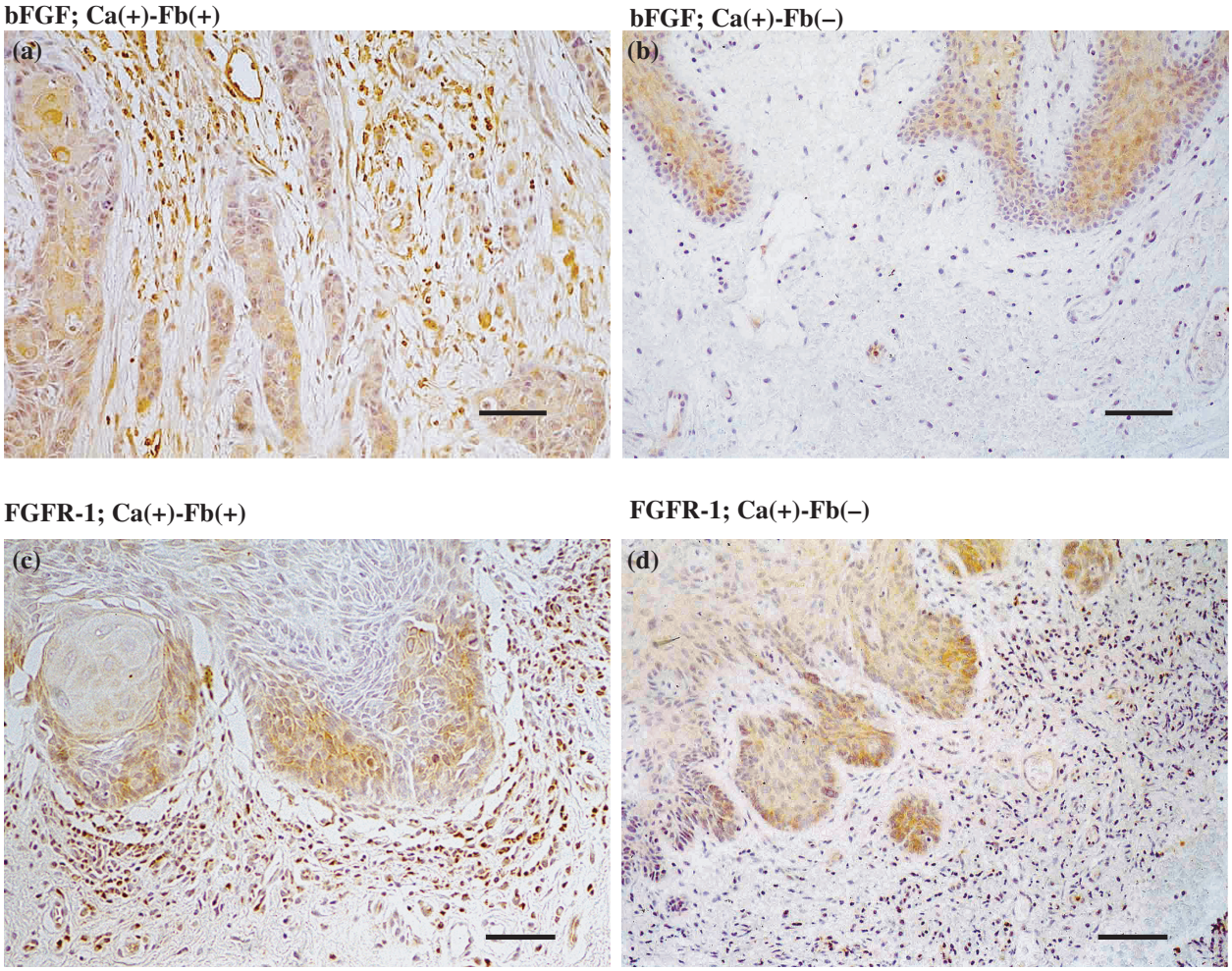

Figure 1 Expression of basic fibroblast growth factor (bFGF) and fibroblast growth factor receptor-1 (FGFR-1) at the invasive front of oral squamous cell carcinoma. Ca(+)-Fb(+): positive expression for both cancer cells and fibroblasts; Ca(+)-Fb(–): positive expression for cancer cells, and negative expression for fibroblasts. Magnification: original ×200 (bar: 100 μm).

Table 2 The relation between bFGF and FGFR-1 expressions and differential type of the OSCC

Differential type	*Number of positive specimens/number of total specimens (%)*	
	bFGF	*FGFR-1*
Well	26/44 (59)	24/44 (55)
Moderate	12/14 (86)	13/14 (93)
Poor	3/3 (100)	2/3 (67)

The prevalence of bFGF and FGFR-1 expression were significantly correlated with differential type of the OSCC ($P < 0.05$; Spearman's correlation coefficient by rank test).
bFGF, basic fibroblast growth factor; FGFR-1, fibroblast growth factor receptor-1; OSCC, oral squamous cell carcinoma.

Table 3 The relation between bFGF and FGFR-1 expressions and mode of invasion of oral squamous cell carcinoma

Mode of invasion (grade)	*Number of positive specimens/number of total specimens (%)*	
	bFGF	*FGFR-1*
1	2/6 (33)	2/6 (33)
2	6/13 (47)	5/13 (39)
3	16/22 (72)	13/22 (59)
4C	10/13 (77)	7/13 (54)
4D	7/7 (100)	7/7 (100)

The prevalence of bFGF and FGFR-1 expression were significantly correlated with mode of invasion ($P < 0.05$; Spearman's correlation coefficient by rank test).
bFGF, basic fibroblast growth factor; FGFR-1, fibroblast growth factor receptor-1.

and/or the fibroblasts for the invasion and growth of the cancer.

In contrast with cancer cells, the fibroblasts did not appear to produce bFGF in all specimens. Amongst specimens with positive bFGF fibroblasts, 83% were also positive for FGFR-1 expression. This confirms the high affinity of FGFR to bFGF, and suggest the presence of receptors for bFGF other than for FGFR-1.

The moderately and poorly differentiated SCC specimens exhibited a higher prevalence of bFGF and FGFR-1 expressions in the fibroblasts compared with

Table 4 The relation between bFGF and FGFR-1 expressions and lymph node metastasis in oral squamous cell carcinoma

	Number of positive case/number of total case (%)	
Lymph node metastasis	*bFGF*	*FGFR-1*
Negative	18/37 (49)	16/37 (43)
Positive	23/24 (96)*	18/24 (75)**

*, **$P < 0.05$, lymph node metastasis negative vs. positive (chi-square test).
bFGF, basic fibroblast growth factor; FGFR-1, fibroblast growth factor receptor-1.

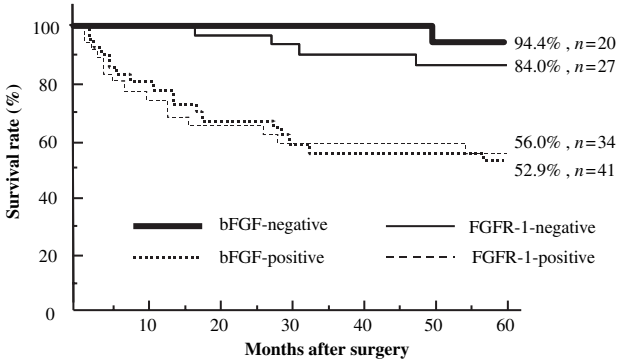

Figure 2 The survival curves of basic fibroblast growth factor/fibroblast growth factor receptor-1 (bFGF/FGFR-1)-positive and -negative patients with oral squamous cell carcinoma ($P < 0.01$; log-rank test).

well-differentiated SCC specimens (Table 2). Moreover, these expressions were well correlated with the mode of invasion (Table 3). Low-grade cancers (grades 1 and 2) are regarded as not so invasive, and high-grade cancers (grades 4C and 4D) are highly likely to have a strong invasion potential (8, 13). It appears that bFGF production by fibroblasts is closely linked to OSCC malignancy. The fibroblasts might produce bFGF for host protection from cancer invasion, or as an inflammation reaction caused by highly malignant cancer. It is also possible that bFGF produced by the fibroblasts plays an important role in cancer invasion. Further study is required to clarify these phenomena.

The expression of bFGF and FGFR-1 in the fibroblasts was obviously correlated with the presence of lymph node metastasis (Table 4). Similar findings were also evident regarding the prognosis of patients (Fig. 2). As the clinical characteristics and prognosis of cancer are closely related to the pathologic characteristics, it is perhaps not surprising that the presence or absence of metastasis and the prognosis of OSCC are linked with bFGF production.

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