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## Reduced Expression of Syndecan-1 in Oral Cancer

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### 1. Introduction

The syndecan family is composed of four closely related proteins (syndecan-1–4) encoded by four different genes. Syndecan-1 binds cells via its heparan sulfate chains to a variety of components of the interstitial matrix, including types I, III and V collagen, fibrillar collagen, fibronectin and tenascin. In previous studies it has been noted that expression of syndecan-1 correlates with malignancy in various tissues including uterine cervix and esophagus. Several reports on head and neck carcinoma have suggested that reduced expression of syndecan-1 is associated with the prognosis of such neoplasms. Anatomical location may influence the expression of syndecan-1 in squamous cell carcinoma (SCC), since previous studies examined this type of cancer in various tissue sites of the oral cavity. Also, previous reports evaluated the immunohistochemical positive ratio ignoring patterns of expression; nevertheless, immunoreaction to syndecan-1 was located only at the cell membranes, and not in the cytoplasm. No study has shown whether or not syndecan-1 is associated with mode of invasion, although invasion correlates to malignant behavior and prognosis.

We investigated syndecan-1 immunoreactivity in primary SCC arising exclusively from the tongue, and the relationship between expression pattern of immunoreactivity and various clinico-pathological parameters was analyzed. Forty-three cases of SCC arising in lateral border of tongue were investigated. From the immunohistochemical staining pattern, the cases were divided into two groups based on expression of syndecan-1 at the supra-peripheral cells of the tumor nest: Group A, completely or mainly positive; Group B, sporadically positive or negative. Most poorly differentiated SCC cases were classified into Group B (81.8%). The number of Group B cases in T1–2 was different from that in T3–4. The number of cases where syndecan-1 expression was reduced was much greater in T3–4, and represented the majority of Group B (86.7%). More than 80% of Grade 4D cases were in Group B (83.3%) based on the Yamamoto–Kohama criteria. These results indicate that reduction of syndecan-1 correlates to histological grading, tumor size and mode of invasion in tongue SCC.

We also investigated the expression of syndecan-1 in oral cancer cell lines and tested whether transfection of an siRNA against human syndecan-1 affected the malignant

potential of these cells. Seven different human oral cancer cell lines (HSC2, HSC3, HSC4, Ca9-22, SAS, KB and BSC-OF) were used. In order to examine syndecan-1 function, siRNA was transfected into the cells, after which the cell growth rate and invasive ability were evaluated. QRT-PCR showed that syndecan-1 was expressed in Ca9-22 cells and that it was significantly higher (>10-fold) than in the other oral cancer cell lines. Transfection of syndecan-1 siRNA was carried out on Ca9-22 cells, which increased their growth rate 1.4-fold above controls. The invasive ability of Ca9-22 cells treated with syndecan-1 siRNA was significantly higher (2-fold; n=5) than the controls. These results suggest that syndecan-1 directly contributes to the growth and invasive ability of these cells.

### 1.1 Syndecan

Cell surface adhesion receptors bind cells to their extracellular matrix and couple such interactions with intercellular signaling mechanisms. It is apparent that alternations in cell adhesion can influence almost every stage of cellular transformation. The development of malignant epithelial neoplasm is associated with disruption of cell-to-cell and cell-to-matrix adhesion.

Syndecans are family of heparan sulfate proteoglycan receptors that are thought to participate in both cell-to-cell and cell-to-matrix adhesion. The syndecans are composed of a core protein, to which sulphated and unbranched carbohydrate chains, glycosaminoglycans, are covalently attached. The core proteins contain an extracellular, a transmembrane and an intracellular domain, and their amino acid sequences are homologous, especially between the two last domains. The syndecans interact with extracellular matrix components, other cell surface components, and growth factors, including basic fibroblast growth factor (Hayashi *et al.*, 1987; Inki *et al.*, 1991). The syndecan family is composed of four closely related proteins; syndecan-1, syndecan-2 (fibroglycan), syndecan-3 (N-syndecan), and syndecan-4 (amphiglycan, ryudocan); encoded by four different genes. Syndecan-1 consists of a 310 amino acid long core protein in human, and is an 85-92 kDa type I integral membrane proteoglycan and binds cells via its heparan sulfate chains to a variety of components of the interstitial matrix, including types I, III and V collagen, fibrillar collagen, fibronectin and tenascin. The syndecan-1 also contains chondroitin sulfacte. The syndecan-1 is thought to function as a matrix receptor that transduces information between the extracellular matrix and the inside of the cell (Inki *et al.*, 1994; Sanderson *et al.*, 1992). Syndecan-1 is expressed in distinct differentiation stages of normal lymphoid cells. During lymphocyte differentiation, syndecan-1 is expressed only when and where lymphoid cells interact with type I collagen (Sanderson *et al.*, 1989), thus it occurs on the cell surface of B cells in the pre-B-cells stage and immature B cells, but is absent from matured B cells, and re-appears on plasma cells (Sebestyen *et al.*, 1999). Sydecan-1 may mediate the adhesion of lymphoid cells to bone marrow matrix and to the interstitial matrix of peripheral lymphoid organs. The most abundant expression of syndecan-1 in the adult organism is found in stratified squamous epithelia, such as epidermis, oral mucosa and vagina. Syndecan-1 is found on basolateral surfaces of the epithelial cells, endothelial cells of sprouting capillaries and embryonic condensing mesenchymal cells (David *et al.*, 1993; Sanderson *et al.*, 1989). In normal tongue tissue, the basal, supra-basal and lower prickle cell layers of the epithelium were immunohistochemically positive for syndecan-1, and positive reactions were usually distinct on the cell surfaces (Figure 1a, 1b). The cell membrane facing the basement

membrane was essentially negative for syndecan-1 staining. The upper prickle cell and superficial layer of the epithelium lacked syndecan-1 reactivity. However, obvious matrix ligands for syndecan-1 are not found within these tissues. Therefore, syndecan-1 may have different functions in stratified epithelia.

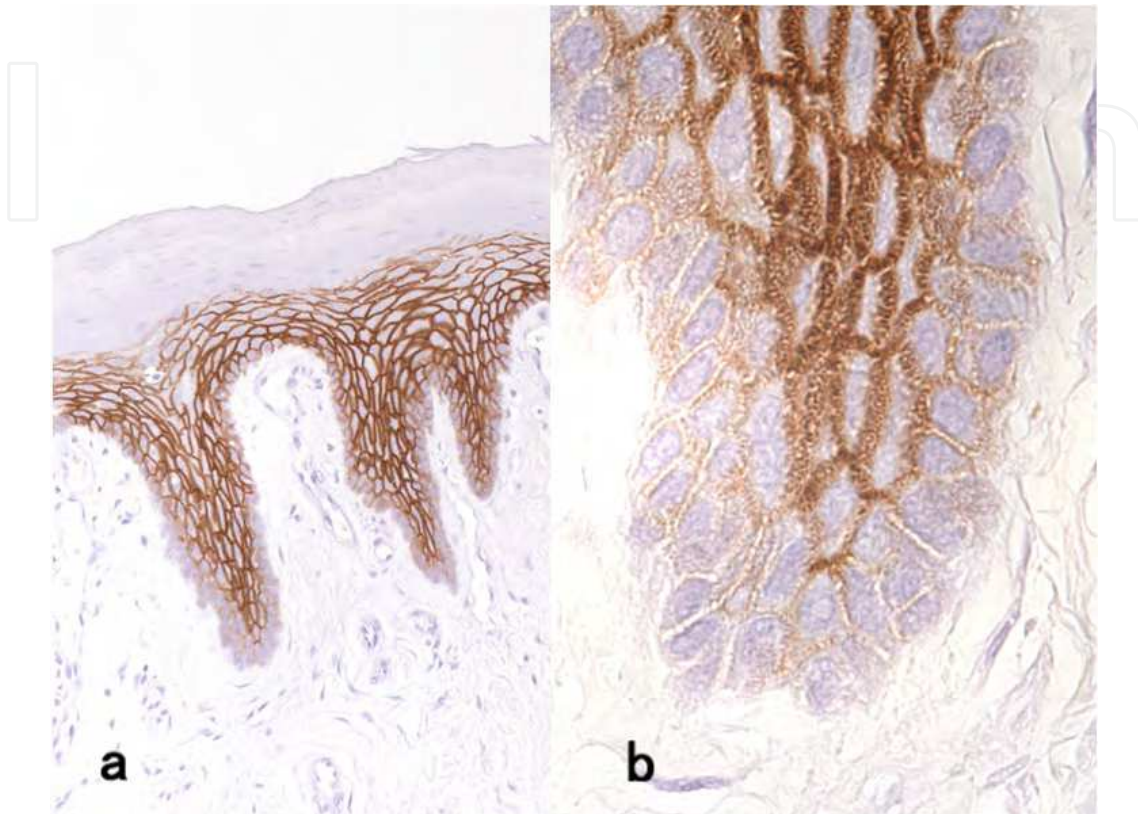


Fig. 1. Immunohistochemical expression pattern of syndecan-1 in normal tongue epithelium. (a) Syndecan-1 expression in the normal tongue. In normal tissues, the basal, suprabasal and lower prickle cell layers of the epithelia are positive for syndecan-1. (b) Higher magnification of (a). The cell membrane facing the basement membrane is essentially negative for syndecan-1. The upper prickle cell and superficial layers of the epithelia lacked syndecan-1 reactivity.

Intracellular tail of syndecan seems to combine the cytoskeleton and cellular components, and the extracellular part of the molecule seems to bind different ligands. Presumably, syndecan-1 plays an important role in cellular functions such as proliferation, cell-to-matrix and cell-to-cell adhesion (Gattei *et al.*, 1999). Interaction of cells and extracellular matrix is important in the maintenance of normal cell architecture and growth, but is especially highlighted during embryonic development involving morphogenetic interactions between different cell types. The expression of syndecan-1 is developmentally highly regulated in a fashion which suggests that syndecan-1 is one of the molecules that participate in reciprocal morphogenetic interactions during embryonic development (Vainio *et al.*, 1991). During mouse tooth development, syndecan-1 is induced by primitive dental epithelium in condensed mesenchyme, but later disappears and becomes localized again in the epithelial cells (Thesleff *et al.*, 1996). Although there have been many investigations, the function of syndecan is still largely unknown.

## 1.2 Syndecan-1 in cancer

In previous studies it has been noted that reduced expression of syndecan-1 correlates to malignancy in various tissues including uterine cervix (Inki *et al.*, 1994; Rintala *et al.*, 1999), endometrium (Miturski *et al.*, 1998), esophagus (Mikami *et al.*, 2001), breast (Barbareschi *et al.*, 2003), lung (Anttonen *et al.*, 2001), kidney (Gokden *et al.*, 2006), liver (Charni *et al.*, 2009), multiple myeloma (Sebestyen *et al.*, 1999). These earlier reports showed that syndecan-1 expression was reduced during malignant transformation of various epithelia (Inki and Jalkanen, 1996). Syndecan-1 was also lost rapidly by myeloma cells entering into apoptosis, thus syndecan-1 is a marker of viable myeloma cells (Jourdan *et al.*, 1998). Many immunohistochemical studies have demonstrated absent or decreased expression of syndecan-1 in many kinds of carcinomas with more aggressive characteristics (Fujiya *et al.*, 2001; Inki *et al.*, 1994; Kumar-Singh *et al.*, 1998; Matsumoto *et al.*, 1997; Stanley *et al.*, 1999; Toyoshima *et al.*, 2001; Wiksten *et al.*, 2000; Wiksten *et al.*, 2001). There are several reports on syndecan-1 expression in head and neck carcinoma (Anttonen *et al.*, 1999; Inki *et al.*, 1994; Kurokawa *et al.*, 2003; Kurokawa *et al.*, 2006; Muramatsu *et al.*, 2008; Ro *et al.*, 2006). However, head and neck carcinoma includes oral, nasal, laryngeal and esophageal carcinomas, and investigation of syndecan-1 expression in oral cancer has been limited.

## 1.3 Syndecan-1 in oral cancer

Oral cancer is the fifth most common type of cancer in the world. Despite modern intervention, the 5-year survival rate for this disease has improved only marginally over the past decade and recurrent disease is observed in 50% of patients (Greenlee *et al.*, 2001). Survival curves of oral cancer patients have plateaued over the past two decades and remain among the worst of all cancer sites (Takes *et al.*, 1997). Recent studies in this field have focused on the development of specific markers that reflect the biological properties of tumors and have use in early detection, disease monitoring and determining the prognosis of patients with oral cancer (Alevizos *et al.*, 2001; Le *et al.*, 2003; Macabeo-Ong *et al.*, 2003).

Syndecan-1 has been reported to be a prognostic factor for tumor progression and survival in various types of malignant tumors, which suggests a close correlation of syndecan-1 expression with malignancy and metastasis (Inki *et al.*, 1994; Kurokawa *et al.*, 2006). In general, transformed cells are often characterized by an abundant secretion of syndecan-1, which results in metastasis formation (Senger *et al.*, 1983; Senger and Perruzzi, 1985). Earlier studies associated syndecan-1 levels with prognosis and have suggested syndecan-1 as a candidate biomarker for the malignant potential of head and neck tumors.

It has been reported that a marked down-regulation of syndecan-1 expression is associated with dysplastic change in the oral epithelium. Kurokawa *et al.* (2003) also found a significant correlation between the down-regulation of syndecan-1 expression and the grade of oral epithelial dysplasia. Down-regulation has also been reported in SCCs of the head and neck compared to expression in the corresponding normal epithelium (Soukka *et al.*, 2000), suggesting that syndecan-1 was a useful marker for evaluating pre-malignant lesions of the head and neck region. In head and neck tumors,

Soukka *et al.* (2000) reported that 65% of oral SCC cases showed negative or weak staining for syndecan-1, of which 35% were totally negative. In our study, 36 of the 72 cases (50%) were negative or weakly intense for syndecan-1 expression. Inki *et al.* (1991) and Soukka *et al.* (2000) reported that intermediate and strong positive staining for syndecan-1 was localized on cell surfaces, especially in cell-cell contact sites. Moreover, Inki *et al.* (1994) demonstrated that syndecan-1 expression was associated with tumor size and histological grade, and tumors with a poor histological grade expressed syndecan-1 at lower levels.

These earlier reports point to reduction of syndecan-1 expression as being a biomarker in head and neck cancer. However, evaluation is different in various studies. For example, anatomical location may influence the expression of syndecan-1 in SCC, since previous studies examined this type of cancer in various tissue sites of the oral cavity such as tongue, maxillary gingiva, mandibular gingiva, oral floor, and buccal mucosa. Also, previous reports evaluated the immunohistochemical positive ratio ignoring patterns of expression; nevertheless, immunoreaction to syndecan-1 was located only at the cell membranes, and not in the cytoplasm.

#### 1.4 Reduction pattern of syndecan-1 in oral cancer

As mentioned above, previous reports evaluated the immunohistochemical positive ratio ignoring patterns of expression. Evaluations of immunohistochemical findings were only reported as negative, weakly positive or positive in previous studies (Kurokawa *et al.*, 2006; Soukka *et al.*, 2000), and the positive ratio was employed frequently as an evaluation of the immunostaining (Anttonen *et al.*, 1999; Inki *et al.*, 1994). Nevertheless, immunoreaction to syndecan-1 was located only at the cell membranes, and not in the cytoplasm of the tumor cells. The evaluation method shown in these earlier reports was vague and not objective. Ro *et al.* (2006) used a new method based on the pattern of immunostaining for syndecan-1. The method was focused on reduced patterns of syndecan-1 in the supra-peripheries, because recent studies had showed that reduction of syndecan-1 expression was associated with proliferative activity (Ki-67 expression) (Kurokawa *et al.*, 2003). Both Ki-67 and syndecan-1 are localized in cells of the supra-basal layer as well, and this was evaluated. This method is considered to be more objective and exact for evaluation than that used in previous studies, because it estimates immunoreactivity restricted to the supra-peripheries of the tumor nests.

From the patterns of immunohistochemical staining, the cases were divided into two groups according to the expression of syndecan-1 as follows. Group A: complete or mostly surround type. Syndecan-1-positive reactions were observed at the supra-peripheral cell layers of the tumor nest without break, or loss of syndecan-1 was seen at within 50% of the supra-peripheral cell layer of the tumor nest (Figure 2a-d). Group B: sporadic expression or negative type. Immunoreactions with syndecan-1 at the supra-peripheral cell layer of the tumor nests were sporadically reduced. Loss of syndecan-1 was seen in 50% or more of the cells (Figures 2e-h). In SCC, positive reactivity for syndecan-1 was usually detected at the supra-periphery of the tumor nests. We divided the cases into two groups according to their immunoreactivity, and the number of cases was 18 (41.8%) in Group A and 25 (58.2%) in Group B.

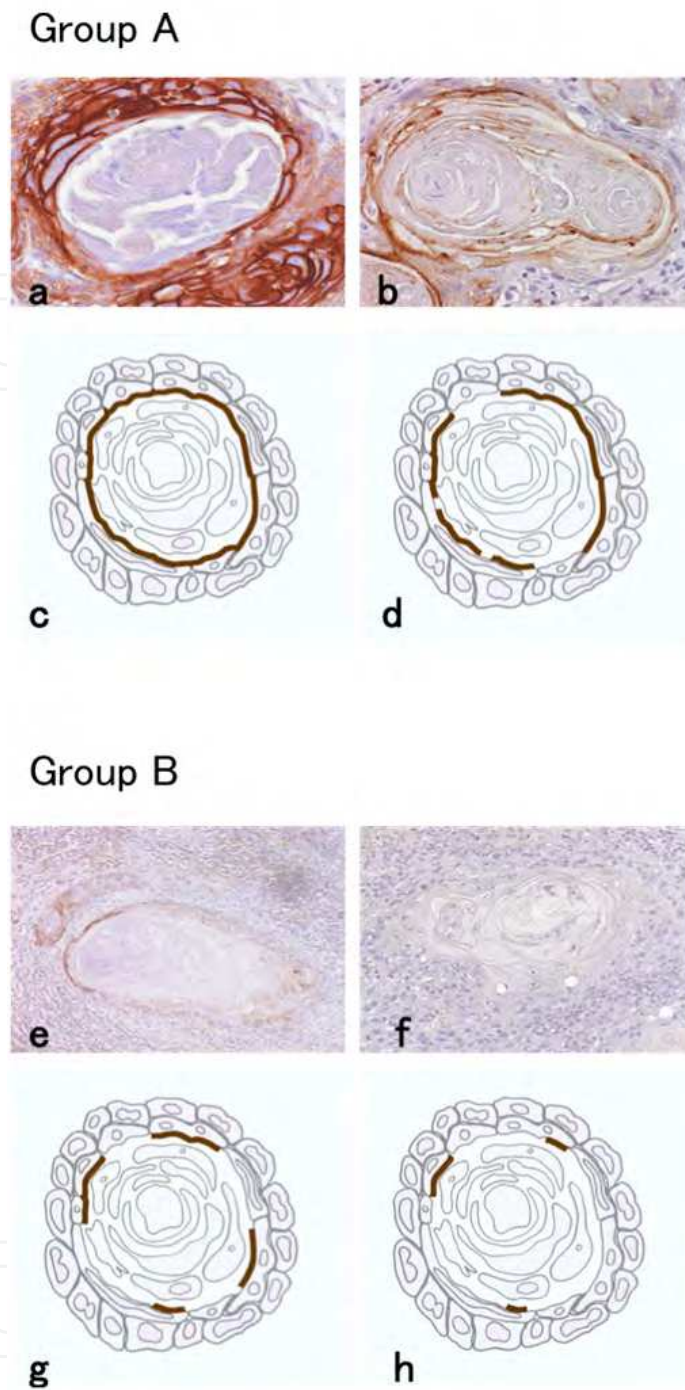


Fig. 2. Immunohistochemical expression pattern of syndecan-1 in SCC of the tongue. We divided pattern of immunohistochemical staining into 2 groups according to the expression of syndecan-1 at periphery of the tumor nest. Group A: Completely (a) or mostly (b) surrounded type. Staining surrounds the tumor nests without a break (a, c). Loss of syndecan-1 staining surrounding the tumor nests is found within 50% of the cells (b, d). Figure (c) and (d) are scheme of figure (a) and (b), respectively. Group B: Sporadically expression (e) or negative type (f). Loss of syndecan-1 surrounding the tumor nests is seen in 50% or more than of the cells (e-h). Syndecan-1 expression is weak (e) or completely negative (f). Figures (g) and (h) are scheme of figure (e) and (f), respectively.

### 1.5 Syndecan-1 expression and clinical parameters

Some investigators demonstrated that syndecan-1 immunoreactivity in primary SCC arising exclusively from the head and neck, especially from the oral cavity, and the relationship between expression pattern of immunoreactivity and various clinico-pathological parameters such as Tumor-Node-Metastasis (TNM) system and histological grading of the differentiation of SCC, was analyzed.

	Group A % (n)	Group B % (n)	
<b>Histological grade</b>			
well	50.0 (12)	50.0 (12)	N.S.
moderately	50.0 (4)	50.0 (4)	N.S.
poorly	18.2 (2)	81.8 (9)	*
<b>Tumor size</b>			
T1 - 2	57.1 (16)	42.9 (12)	N.S.
T3 - 4	13.3 (2)	86.7 (13)	*
<b>Stage</b>			
stage I - II	50.0 (7)	50.0 (7)	N.S.
stage III - IV	37.9 (11)	62.1 (18)	N.S.
<b>Mode of invasion</b>			
Grade 1	50.0 (1)	50.0 (1)	N.S.
Grade 2	54.5 (6)	45.4 (5)	N.S.
Grade 3	40.0 (6)	60.0 (9)	N.S.
Grade 4C	37.5 (3)	62.5 (5)	N.S.
Grade 4D	16.7 (1)	83.3 (5)	*
<b>Invasion depth</b>			
0 - 3 mm	27.3 (3)	72.7 (8)	*
3 - 6 mm	33.3 (5)	66.7 (10)	N.S.
6 - 9 mm	62.5 (5)	37.5 (3)	N.S.
9 mm -	50.0 (5)	50.0 (5)	N.S.
<b>Lymph node metastasis</b>			
pN0	35.7 (5)	64.3 (9)	N.S.
pN1	40.0 (4)	60.0 (6)	N.S.

N.S.: not significant

\*: statistically significant (p<0.01)

Table 1. Correlation between reduction of syndecan-1 and clinicopathological parameters



Ro *et al.*, (2006) investigated forty-three cases of SCC arising in lateral border of tongue (**Table 1**). For histological grade, a reduction in syndecan-1 staining was apparent in poorly differentiated SCC (81.8%), while in well or moderately differentiated SCCs, there was no difference. Regarding tumor size, the number of sporadically positive or negative syndecan-1 cases in T1 or T2 SCC was different from that in T3 or T4. Reduction in syndecan-1 expression was dramatically greater in T3 and T4, and such cases represented the majority of negative syndecan-1 cases (86.7%). Concerning stage, the incidence of syndecan-1-negative cases in stages I and II was different from that in stages III and IV. Sixty-two percent of stages III and IV cases were in of negative syndecan-1 cases, suggesting that advanced SCC shows greatly reduced expression of syndecan-1. However, there was no correlation between reduction of syndecan-1 and depth of invasion or lymphatic metastasis. The results of the earlier studies (Inki *et al.*, 1994; Kurokawa *et al.*, 2006; Ro *et al.*, 2006) suggest that reduction of syndecan-1 expression is correlated with tumor size, histological grade and mode of invasion in SCC of tongue as well as other body sites (Anttonen *et al.*, 2001; Kumar-Singh *et al.*, 1998), but is not associated with lymph-node metastasis and depth of invasion, which are accurate predictors of clinical outcome. There has been controversy regarding the relationship between lymphatic metastasis and syndecan-1 expression. Anttonen *et al.* (1999) noted that strong syndecan-1 expression was associated with lack of lymph-node metastasis, but Inki *et al.* (1994) demonstrated that there was no association between syndecan-1 expression and the presence of cervical node metastasis, the results of the present study being in agreement with Inki *et al.* (1994). It is proposed that while syndecan-1 may be a useful candidate biomarker, it is not an accurate predictor of clinical outcome in SCC of tongue.

### 1.6 Syndecan-1 on oral SCC invasion

Oral cancer is characterized by a high degree of invasion into local tissues. The mode of invasion of the malignant tumor is an important factor in predicting prognosis, and has been studied in head and neck tumors in particular (Jakobsson *et al.*, 1973; Yamamoto *et al.*, 1983). Recent evidence suggests that cells present at the invasive tumor front of carcinomas have different molecular characteristics compared with those in superficial areas of the tumor, making the invasive front the most important area of the tumor for determining the prognosis (Bryne *et al.*, 1989; Bryne, 1991; Bryne *et al.*, 1992). Bryne *et al.* (1995) described a multiple-factor histological grading system of the invasive front of tumors of the head and neck: it consisted of the pattern of invasion, the degree of keratinization, nuclear polymorphism, and the host response. Kearsley and Thomas (1993) reported a strong correlation between total malignancy grading scores based on several pathological parameters and the prognosis in oral SCC. Kurokawa *et al.* (2006) evaluated the association between the loss of syndecan-1 expression and the histological grade of malignancy at the deep invasive front in oral SCC using the method of Bryne *et al.* (1992). and reported a statistically significant correlation between the down-regulation of syndecan-1 expression and prognosis, differentiation and pattern of invasion at the deep invasive front in oral SCC (Kurokawa *et al.*, 2006).

On the other hand, there has been reported classification on mode of invasion by Yamamoto *et al.* (1983), named as Yamamoto-Kohama's criteria (**Table 2**). They have been widely utilized and considered useful for estimating risk factors (Yamamoto *et al.*, 1983). The Grade 4C oral SCC is characterized by a cord-like, diffuse, deep invasion forming cord-shaped micro-tumor nests. Grade 4D oral SCC invades the deeper portion diffusely as a single cell or a few cells.

The study by Ro *et al.* (2006) employed Yamamoto–Kohama's mode-of-invasion criteria, and the correlation between mode of invasion and reduction of syndecan-1 expression was demonstrated by diffuse invading SCC having only faint expression of syndecan-1. More than 70% of Grades 4C and 4D were classified into syndecan-1 reduced or negative cases (71.4%) according to Yamamoto–Kohama's criteria. Expression of syndecan-1 has been known to suppress the level of matrix metalloproteinase (MMP)-9 and to inhibit cell invasion into type I collagen (Kaushal *et al.*, 1999; Liebersbach and Sanderson, 1994). Syndecan-1 is degraded by heparanase (Reiland *et al.*, 2004), and mode of invasion is associated with MMPs (P *et al.*, 2001) and heparanase activity (Ikebe *et al.*, 1999). These reports suggest that reduced expression of syndecan-1 is strongly associated with the mode of invasion.

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Grade

1. Well-defined borderline
  2. Cords, less marked borderline
  3. Groups of cells, no distinct borderline
  4. Diffuse invasion
    - 4C: Cord-like type
    - 4D: Widespread type
- 

Yamamoto–Kohama's classification (1983)

Table 2. Histological grading of mode of invasion

## 2. Studies in oral cancer cells

Recent *in vitro* studies have indicated that syndecan-1 plays a role in inhibiting cell invasion and suppressing the growth of carcinoma cell lines (Ito *et al.*, 2003; Liebersbach and Sanderson, 1994; Liu *et al.*, 1998; Mali *et al.*, 1994).

Our earlier study showed that the reduction of immunoreactivity for syndecan-1 in oral SCC cells was associated with tumor size, suggesting that syndecan-1 contributes to their malignant behavior including changes in growth and invasive ability (Ro *et al.*, 2006). A reduction of syndecan-1 expression was associated with proliferative activity (Ki-67 expression) (Kurokawa *et al.*, 2003). Furthermore, Su *et al.* reported that shedding of syndecan-1 by stromal fibroblasts stimulated the proliferation of human breast cancer cells via activation of FGF2 (Su *et al.*, 2007). However, expression levels and function(s) of syndecan-1 in oral cancers have not been clarified. Muramatsu *et al.*, (2008) investigated the expression of syndecan-1 in oral cancer cell lines and tested whether transfection of an siRNA against human syndecan-1 affected the malignant potential of these cells. Seven different human oral cancer cell lines (HSC2, HSC3, HSC4, Ca9-22, SAS, KB and BSC-OF) were used. In order to examine syndecan-1 function, siRNA was transfected into the cells, after which the cell growth rate and invasive ability were evaluated. To validate the high expression levels of syndecan-1 in human oral cancer cell lines, QRT-PCR was carried out. Based on the  $\Delta\Delta C_t$  relative to KB cells, the relative expression levels of syndecan-1 mRNA in oral carcinoma cell lines were calculated. Several cell lines showed expression of syndecan-1 at high levels. In particular, syndecan-1 was expressed in Ca9-22 cells at a higher level (13.2-fold) than in KB cells. Immunofluorescence analysis showed that positive reactions for syndecan-1 were observed strongly at the cell membrane of Ca9-22 cells, while diffuse faint

dot reactions were seen in KB cells. Based on those QRT-PCR and immunofluorescence results, we used Ca9-22 cells as a model for high expression of syndecan-1 as a model cell line. In order to characterize the effects of syndecan-1 siRNA, we carried out QRT-PCR analysis using mRNAs from non-transfected (control) and from transfected Ca9-22 cells. After 48 h of incorporation, decreased expression (1/10-fold) of syndecan-1 was seen in the siRNA-transfected cells, suggesting that siRNA blocked syndecan-1 expression very efficiently in Ca9-22 cells. Cell growth. To examine whether syndecan-1 is associated with cell growth, the growth of siRNA-transfected cells and control cells was measured. Ca9-22 cells had increased growth after syndecan-1 siRNA transfection. The numbers of siRNA-transfected Ca9-22 cells and control cells at 48 h were  $6.0 \times 10^5$  and  $4.1 \times 10^5$ , respectively and at 72 h were  $11.8 \times 10^5$  and  $8.0 \times 10^5$ , respectively. The cell growth rate of control cells was lower than the siRNA-transfected cells at 48 and at 72 h, and were significantly different at both time points ( $p < 0.01$ ). Invasion assay. To examine the effect of syndecan-1 on the invasive ability of Ca9-22 cells, a Matrigel assay was used. The numbers of invasive siRNA-transfected and control Ca9-22 cells were 718.4 per  $\text{mm}^2$  and 378.8 per  $\text{mm}^2$  (average), respectively, which was significantly higher ( $p < 0.01$ ) (**Figure 3**). Our results show that the reduction of syndecan-1 function by siRNA leads to higher levels of cell proliferation, which suggests that syndecan-1 is directly associated with cell proliferation. Furthermore, cell migration has been reported to influence invasiveness and to be an important factor in the incidence of metastasis.

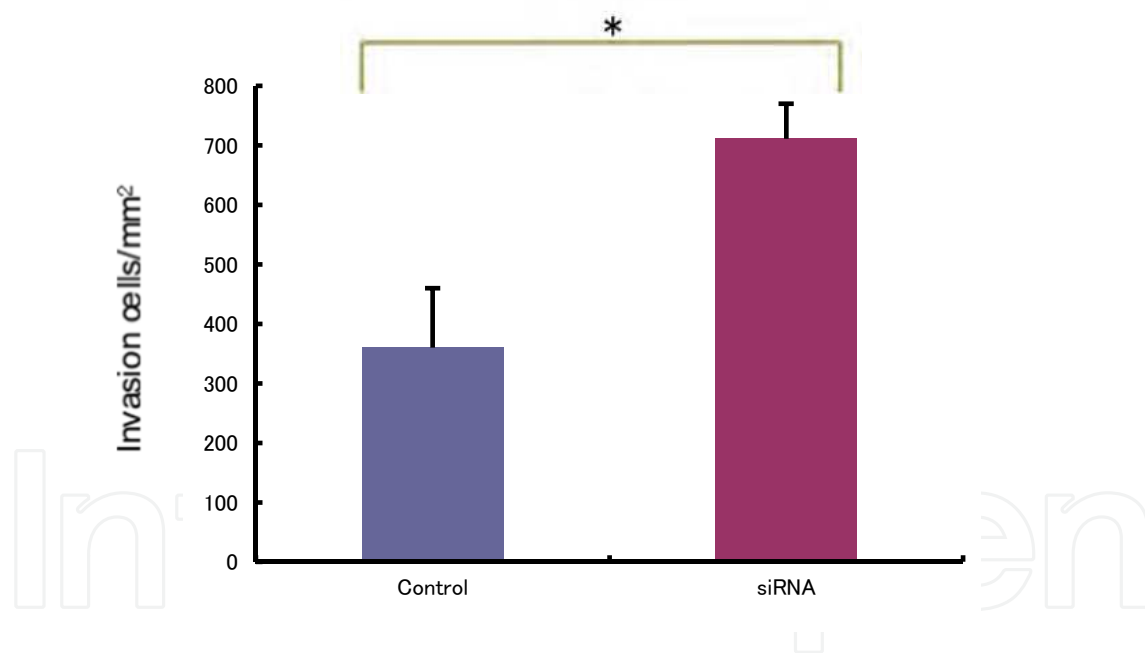


Fig. 3. Invasion assay.

The Matrigel assay was used to investigate the effects of syndecan-1 on the invasive ability of Ca9-22 cells. The number of invading cells was significantly higher in the siRNA-transfected group (\*  $P < 0.01$ ).

Moreover, the invasive ability of tumors is closely related to the incidence of metastasis and the prognosis of the disease. As shown in previous studies, reduced expression of syndecan-1 correlates with metastasis of various tumors. There have been only a few studies that showed a correlation between syndecan-1 and invasion in oral cancers, but there has been no previous functional study of syndecan-1 using an oral cancer cell line. Therefore, we examined whether

syndecan-1 was associated with the invasive ability of Ca9-22 cells. Our results show that invasiveness increased when syndecan-1 function was blocked in siRNA-transfected Ca9-22 cells. The expression of syndecan-1 is known to suppress the level of matrix metalloproteinase (MMP)-9 and to inhibit cell invasion into type I collagen (Kaushal *et al.*, 1999; Liebersbach and Sanderson, 1994). Moreover, syndecan-1 can be degraded by heparanase (Reiland *et al.*, 2004), and invasion is associated with MMPs (O-Charoenrat *et al.*, 2001), and heparanase activities (Ikebe *et al.*, 1999). The syndecan-1 siRNA may induce MMPs and heparanase activity and thus reduce the expression of syndecan-1 in Ca9-22 cells.

### 3. Conclusion

The results of earlier studies, taken together with our studies, suggest that syndecan-1 is a candidate for being a useful biomarker, but is not an accurate predictor of clinical outcome in oral SCC, and directly contributes to the growth and invasive ability of oral cancer cells.

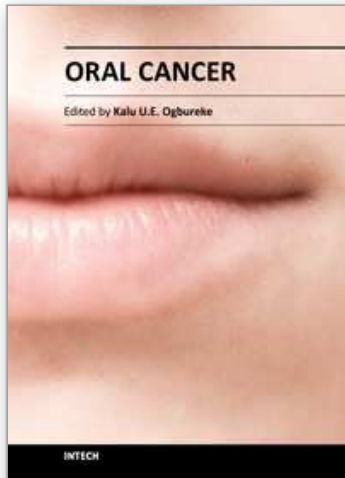
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Oral cancer is a significant public health challenge globally. Although the oral cavity is easily accessible, early diagnosis remains slow compared to the enhanced detection of cancers of the breast, colon, prostate, and melanoma. As a result, the mortality rate from oral cancer for the past four decades has remained high at over 50% in spite of advances in treatment modalities. This contrasts with considerable decrease in mortality rates for cancers of the breast, colon, prostate, and melanoma during the same period. This book attempts to provide a reference-friendly update on the etiologic/risk factors, current clinical diagnostic tools, management philosophies, molecular biomarkers, and progression indicators of oral cancer.

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