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ORIGINAL ARTICLE

Serum soluble CD44v6 levels in patients with oral and maxillofacial malignancy

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OBJECTIVE: To determine the levels of serum sCD44v6 in patients with oral cancer and evaluate the value of serum sCD44v6 in adjuvant diagnosis, staging and monitoring treatment response in these patients.

MATERIALS AND METHODS: A total of 112 hospitalized patients with oral and maxillofacial malignancy and 28 healthy individuals were examined for serum sCD44v6 levels. Venous blood was collected from these patients and the healthy individuals. One week after treatment, venous blood was collected once again in 60 patients with oral and maxillofacial squamous cell carcinoma (OSCC). RESULTS: The sCD44v6 concentration was not significantly different between patients with oral and maxillofacial malignancy and control group ($P > 0.05$). The levels of serum sCD44v6 in patients with OSCC and salivary carcinoma showed no difference with those in control group ($P > 0.05$). The sCD44v6 level in patients with stage III and IV disease was higher than that of patients with stage I and II and that of the control group, but the difference was not significant ($P > 0.05$). Serum sCD44v6 levels in patients with OSCC after treatment became lower than that prevailed during pretreatment ($P < 0.05$). CONCLUSION: The possible roles of CD44v6 in the diagnosis of oral and maxillofacial malignancy deserve further elucidation and evaluation. Serum sCD44v6 may be a valuable marker in monitoring treatment response in patients with OSCC.

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Keywords: CD44v6; oral cancer; serum

Introduction

It has been known that CD44 is a kind of adhesion molecule, which is important for tumour proliferation and metastasis.In recent years, researchers have found that CD44 expresses at abnormally high levels in carcinoma tissues such as in gastric malignancy, nasopharyngeal neoplasm and pancreatic carcinoma. Moreover, studies have also shown that level of serum soluble CD44v6 (sCD44v6), which expresses in patients with gastric malignancy, colon cancer or breast carcinoma, can be potentially used as an indicator of tumour burden and metastasis. However, few studies have been undertaken to determine whether sCD44v6 levels in serum of patients can be a marker of oral and maxillofacial cancer and metastasis. In this study, serum levels of sCD44v6 were measured with quantitative enzyme-linked immunosorbent assay (ELISA) in 112 patients with oral and maxillofacial malignancy before and after treatment and in 28 healthy individuals.

Materials and Methods

Serum samples collection

A total of 112 hospitalized patients (64 men and 48 women) with oral and maxillofacial malignancy were included. The diagnosis of malignancy was confirmed in each case by a pathology report based on histological examination. The patients aged between 29 and 80 years with a mean of 56.34. The lesions included oral and maxillofacial squamous cell carcinoma (OSCC) (68 cases), salivary adenoid cystic carcinoma (ACC) (24 cases), and salivary mucoepidermoid carcinoma (12 cases), melanoma in palate and mandibular gingiva (five cases) and osteosarcoma in mandible (three cases). The 104 patients (including 68 cases of OSCC and 36 cases of salivary gland carcinoma) were staged according to Union Internationale Contre le Cancer (UICC) recommendations (2002, for oral cancer and salivary gland carcinoma) and scheduled for treatment by surgery, chemotherapy or a combination of the two modalities. Twenty-eight healthy individuals aged between 45 and 77 years with a mean of 58.34 served as control. Venous blood was collected from the healthy individuals and these patients before therapy. One week after therapy, venous blood was collected once again in 60 patients with OSCC.

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Determination of serum sCD44

Serum samples were obtained by centrifugation (986 *g*) and stored at −80°C until assayed. Serum levels of sCD44v6 were measured with quantitative enzyme-linked immunosorbent assay (ELISA) with commercially available kits made by GeneMay Company in San Diego, CA, USA. The test was performed according to the manufacturer's instructions. The apparatus to perform ELISA assay is named Rain-Bow 340–700 and made in Anthos, Salzburg, Austria. The software used to deal with statistical data is SAS system for Windows v6.12 (SAS Institute Inc., Cary, NC, USA). Serum levels of sCD44v6 were expressed in ng ml^{-1}. The Student's test was used to assess the significance of difference in levels of serum sCD44v6 between two groups of samples and pair Student's test to compare the serum levels in patients with OSCC before and after treatment, and 95% confidence interval was determined for the difference. Serum sCD44v6 levels in different stages of disease in 104 untreated cancer patients were analysed using analysis of variance (ANOVA).

Results

Serum CD44 levels

The concentrations of sCD44v6 levels in serum of 28 age- and gender-matched normal controls and 112 patients before therapy were 189.19 ± 48.13 ng ml^{-1} and 192.22 ± 75.18 ng ml^{-1} respectively (Table 1). No statistical differences were seen in sCD44v6 concentrations between the patients with oral and maxillofacial malignancy and normal controls ($P > 0.05$), and between squamous cell carcinoma patients and controls ($P > 0.05$). Serum sCD44v6 levels in salivary adenoid cystic carcinoma and salivary mucoepidermoid carcinoma patients showed no difference with that in controls ($P > 0.05$).

Comparison of serum CD44 levels between different stages

The 104 oral cancer patients were grouped according to the clinical stage of the disease and serum sCD44v6 levels were compared in Table 2. Before therapy, serum concentration of sCD44v6 was higher in patients with stage III and IV than in patients with stage I and II. However, the difference was not significant ($P > 0.05$). Also, there was no significant difference in sCD44 values between stages I and II nor between stages III and IV ($P > 0.05$).

Table 1 Serum sCD44v6 levels of controls and untreated patients with oral and maxillofacial malignancy

Group	*No of cases*	*mean ± s.d (ng ml^{-1})*
Control	28	189.19 ± 48.13
Oral and maxillofacial malignancy	112	192.22 ± 75.18
OSCC	68	181.74 ± 73.76
Salivary ACC	24	221.93 ± 104.58
Salivary mucoepidermoid carcinoma	12	220.91 ± 100.32
Melanoma and sarcoma	8	211.38 ± 80.68

Table 2 Serum sCD44v6 levels in 104 untreated cancer patients according to stage of disease

Group	*No of cases*	*mean ± s.d (ng ml^{-1})*
Normal	28	189.19 ± 48.13
Stage I	40	171.36 ± 29.58
Stage II	25	173.43 ± 30.88
Stage III	24	223.23 ± 75.97
Stage IV	15	225.58 ± 110.82

CD44 levels before and after treatment

We compared the concentration of serum sCD44v6 in 60 patients with OSCC before and after treatment. Through pair Student's test, serum sCD44v6 levels in patients with OSCC after complete treatment became lower than that prevailing before treatment ($P < 0.05$), and the Pair Student's test results were $t = 2.396$, $P = 0.0311$, mean $d = -27.70$, s.e. $= 11.563$.

Discussion

CD44 is a transmembrane glycoprotein that binds hyaluronan, extracellular matrix proteins and growth factors. Alternative splicing of a single gene generates a family of splice variants (CD44vl-10) in addition to the standard form (CD44s). Expression of the variable exons (such as v6, v9) has been correlated with tumour progression and metastasis in a range of cell types. However, multiple CD44 isoforms are also expressed in normal stratified squamous epithelia, such as the epidermis and the lining of the oral cavity.

In recent literatures, different results have been reported in different organs with tumour burden about the expression of CD44v6. For example, abnormal high level expression of CD44v6 was found in the tissues of gastric cancer, nasopharyngeal cancer, and pancreatic cancer (Heider *et al*, 1993; Su *et al*, 2000; Rall and Rustgi, 1995). However, abnormal low level expression of CD44v6 existed in the tissues of oral squamous cell carcinoma (Kunishi *et al*, 1997). Studies have also found that in patients with breast carcinoma, gastric or colon cancer, CD44v6 expressing at high levels in cancer tissue accompanies an elevated sCD44v6 level in serum when metastasis occurs (Mayer *et al*, 2008). The elevated level of serum sCD44v6 decreased significantly after the tumours were dissected by surgery (Guo *et al*,1994; Ming *et al*,1999). These results indicate that the concentration of serum sCD44v6 is correlated with tumour burden and metastasis occurrence. Thus, the sCD44v6 levels in serum of patients with these cancers may be used as a guide in monitoring patients' response, detecting metastasis and in predicting prognosis. Kawano found that the higher serum level of sCD44v6 was significantly associated with distant metastasis. Especially, they found that the pretreatment serum levels of sCD44v6 were markedly associated with TNM staging (Kawano *et al*, 2005a, b). In another investigation, CD44 was highly expressed in gastric adenocarcinoma and correlated with a poor prognosis in patients with the intestinal type of gastric adenocar-

cinoma. CD44 can, therefore, be utilized as a prognostic marker for this group of patients (Ghaffarzadehgan *et al*, 2008). However, other studies show that there was no significant difference in serum levels of sCD44v6 between head and neck cancer patients and healthy smokers, and nor was there a correlation between the serum level of sCD44v6 and UICC stage, TNM stage or histological grading (Andratschke *et al*, 2005).

Soluble CD44v6 present in the blood circulation of patients most likely comes from tumour cells rather than normal cells. CD44v6 expresses at a high level in normal oral mucosa, but expresses at low level in OSCC tissue. Also, significant abnormal low level in tumour tissue of OSCC patients often indicates lymph node metastasis. The poorer of the tumour differentiated, the lower of the concentration of the patients' serum sCD44v6 (Kunishi *et al*, 1997).

Later studies show that CD44v6 expresses positively in all normal salivary tissue and in 70% ACC tissue (Wu *et al*, 1999). Some investigators reported that there is no significant difference between the expressions of low grade malignant minor salivary tumour and high-grade malignant salivary ACC, and that the expression of CD44v6 is not related with the degree of malignancy of the tumour (Xing *et al*, 1998).

We have found in this study that the sCD44v6 levels in serum of patients with oral and maxillofacial malignancy did not show significant change compared with normal individuals, and the concentration of sCD44v6 in serum of patients with oral and maxillofacial malignancy with stage I and II were not significantly different from those with stage III and IV.

The results of this study indicate that serum sCD44v6 levels in patients with OSCC after a complete treatment became lower than before treatment ($P < 0.05$), suggesting that correlation of changes in serum sCD44v6 levels with treatment.

We conclude from this study that the determination of serum sCD44v6 appear to be of little value in diagnosis of oral and maxillofacial malignancy and in staging. However, the present study suggests that determination of serum sCD44v6 levels may be of value in evaluating treatment effect in oral and maxillofacial malignancy.

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Author contributions

RD Xing designed the study, SM Chang performed the experiment and wrote the text of this manuscript. FM Zhang and YQ Duan contributed to the collection of data.

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