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Inhibition of osteopontin expression and function in oral cancer cell lines by antisense oligonucleotides

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Abstract

We examined expression and function of osteopontin (OPN) in oral cancer cell lines using antisense oligonucleotide (AS). Quantitative real-time RT-PCR showed that expression in BSC-OF cells was significantly higher (10-fold) than that in KB cell. AS-study showed that foci of AS-treated BSC-OF cells possessed thin processes and radiated morphologically, although BSC-OF cells showed round foci. Cell growth in AS-group was lower ($<80\%$) than the control. Invasion ability in AS-group became significantly lower ($P<0.01$). These results suggest that BSC-OF cell is useful for over-expression of OPN, and that OPN contributes to morphology, growth and invasion.

Keywords: Oral cancer; Extracellular matrix; Invasion; Osteopontin; Basaloid squamous cell carcinoma; Antisense

1. Introduction

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 [1] and recurrent disease is observed in 50% of the patients [2]. Survival curves of oral cancer patients have plateaued over the past two decades and remain among the worst of all cancer sites. Therefore, recent studies in the field have focused on the development of biomarkers for early detection, disease monitoring and determining prognosis of patients with oral cancer [3–5].

Osteopontin (OPN) is a sialic acid-rich glycophosphoprotein that contains an RGD cell-binding sequence; it is a non-collagenous protein that plays an important role in development and remodeling of bone tissue [6–8]. Several studies have shown that OPN is expressed not only in hard tissues but also in other soft tissues including kidney, mammary gland

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and salivary gland [9,10]. Particularly OPN is highly expressed in kidney, suggesting that it inhibits calcification. OPN has also been shown to be multifunctional, with activities in cell migration, cell survival, inhibition of calcification, regulation of immune cell function, and control of tumor phenotype [11]. Recently it has been reported that OPN is a prognostic factor for tumor progression and survival in a number of solid neoplasms including breast, gastric, brain, ovary and prostate, suggesting a correlation between OPN expression/function with malignancy and metastasis [8,11–13]. It has also been suggested that OPN might be a candidate biomarker for malignancy [3,13]. In the head and neck region, over-expression of OPN was found only in dysplastic and squamous cell carcinomas (SCC) and not in normal mucosa [14]. However, expression levels and function(s) of OPN in oral cancers remain unknown. In the present study, we examined OPN expression in a number of oral cancer cell lines and antisense (AS) oligonucleotides were used to characterize the function(s) of OPN in these cells.

2. Materials and methods

2.1. Cell cultures

Human oral SCC cell lines, HSC2, HSC3, HSC4, SAS and KB, were obtained from the Japanese Cancer Research Resources Bank (JCRB). BSC-OF was established from an oral basaloid squamous cell carcinoma by Abiko et al. [15]. All cell lines were grown in Dulbecco's modified Eagle medium (DMEM) or DMEM/F-12 (Invitrogen, Grand Island, NY, USA) containing 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO, USA) and penicillin–streptomycin (100 U/ml, invitrogen) at 37 °C in humidified incubator containing 5% CO_2.

2.2. RT-PCR and quantitative real time RT-PCR (QRT-PCR)

Total RNA was extracted using the acid guanidinium–thiocyanate–phenol–chloroform (AGPC) method with TRIzol (Invitrogen) and cDNA synthesis was performed as described in our previous reports [16,17]. For analysis of OPN gene expression by RT-PCR, RNA was reverse-transcribed into complementary DNA (cDNA) using a Takara RNA PCR Kit (Takara, Tokyo, Japan). The sequences of the primers were specific, as confirmed by a computer-assisted search of an updated version of GenBank. Primer sequences used to detect human OPN and β-actin (as an internal control) are shown in Table 1. In each tissue sample, 100 ng total RNA was reverse transcribed using random primers and the products were subjected to PCR amplification under the same conditions described above. The reaction mixture was added to the RNA solution, incubated at 42 °C for 1 h, heated at 94 °C for 5 min, and then chilled at 4 °C. For PCR, the cDNA reaction mixture was diluted with 40 μl PCR buffer and mixed with 50 pmol of the 5′ and 3′ primers. Reactions were carried out in a DNA thermal cycler (MJ Research Inc., Watertown, MA, USA) under the following conditions: 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min. The amplified products were then analyzed by 1.7% agarose gel electrophoresis and visualized by ultraviolet illumination after staining with ethidium bromide.

QRT-PCR was carried out according to Kim et al. and Ohtsuka et al. with modification [13,18]. For QRT-PCR studies, a total 2 μl cDNA was used in a 23-μl PCR mixture containing 1× SYBR PCR buffer, 3 mM $MgCl_2$, 0.8 mM dNTP and 0.025 U/μl AmpliTaq Gold DNA polymerase (PE Applied Biosystems, Foster City, CA, USA). We used primers for human OPN and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control. The sequences are shown in Table 1. Amplification was then performed in duplicate using the primer sets in an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems) with denaturation

Table 1
Sequences of primers used in the present study

For RT-PCR	
Osteopontin	F: 5′-TTG CTT TTG CCT CCT AGG CA-3′
	R: 5′-GTG AAA ACT TCG GTT GCT GG-3′
β-actin	F: 5′-TGT ATG CCT TCG GTC GTA CCA C-3′
	R: 5′-ACA GAG TAC TTG CGC TCA GGA G-3′
For QRT-PCR	
Osteopontin	F: 5′-GTT GCA GCC TTC TCA GCC AA-3′
	R: 5′-GCA AAA GCA AAT CACTGC AAT T-3′
GAPDH	F: 5′-GTC ATG GGT GTG AAC CAT GAG AA-3′
	R: 5′-TGG TCA TGA GTC CTT CCA CGA T-3′