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Original Research Article

Visfatin/pre-B cell colony-enhancing factor immunohistochemical overexpression in oral cancers

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ABSTRACT

Increased visfatin expression has been shown to increase gene expression, which promotes cell survival and increases SirT1 activity thereby promoting angiogenesis. Previous studies have shown that oral squamous cell carcinomas (OSCCs) express high levels of activated signal transducer and activator of transcription 3 (Stat3). Since visfatin expression is increased by Stat3, we hypothesized that visfatin protein may be highly expressed in OSCCs. Immunohistochemistry was the technique used to examine the expression of visfatin in 19 OSCCs and 4 hyperplastic lesions. The results indicated that visfatin was detected in the cytoplasm and nuclei of the OSCCs and epithelial hyperplasia as well as in the stromal tissues of patients with OSCC and oral hyperplasia. Furthermore, co-expression of visfatin and proliferating cell nuclear antigen proteins was noted in verrucous epithelial hyperplasia, and co-expression of visfatin and CD68 in the inflammatory cells of the stromal region was noted in the OSCCs. In addition, enzyme-linked immunosorbent assay showed that plasma visfatin concentrations were significantly increased in the patients with OSCC and oral hyperplasia compared to those of the control subjects. In conclusion, visfatin expression and concentrations were higher in OSCCs and oral hyperplasia, suggesting that visfatin may play a role in the pathogenesis of oral cancers.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity and the eighth most common cancer in the world (Massano et al., 2006). In Taiwan, oral cancers ranked as the sixth most prevalent cancers in both sexes and the fourth most common cancers in males in 2006 (Cancer Registry Annual Report in Taiwan Area, 2007). Therefore, it is critical to discover prognostic factors as well as therapeutic targets for oral cancers. Visfatin, also known as pre-B cell colony-enhancing factor, catalyses the rate limiting step of nicotinamide adenine dinucleotide (NAD+) synthesis (Garten et al., 2009). Visfatin expression promotes cell growth and survival and angiogenesis, and has been shown to be highly expressed in ovarian cancers, gastric and colorectal carcinomas, and malignant astrocytomas/glioblastomas (Hufton et al., 1999; Van Beijnum et al., 2002; Yang et al., 2007; Reddy et al., 2008; Garten et al., 2009; Nakajima et al., 2009; Shackelford et al., 2010). It has also been shown that visfatin may be induced (~1.5 to 2.0-fold above basal levels) by factors such as hypoxia, serum deprivation, and methylmethane sulfonate in cell cultures, and fasting in animals, via a signal transducer and activator of the transcription 3 (Stat3)-dependent mechanism (Nowell et al., 2006; Yang et al., 2007). Stat3 is a transcription factor that has been shown to act as an oncogene in several human malignancies including oral cancer (Bowman et al., 2000; Zhao et al., 2012). In addition, our recent study suggested that visfatin may act through inflammatory reactions to play an important role in the pathogenesis of OSCC (Tsai et al., 2013). As biomedicine is an interdisciplinary area connecting human medicine in every field, it involves the study of patho-physiological processes using the methods of modern biology and being an important way to investigate human diseases, particularly from the perspective of devising new strategies for diagnosis and therapy (Berger, 2011). The aim of this study was to further substantiate a potential role for visfatin in oral cancer by studying visfatin protein expression patterns in patients with oral cancer and precancerous tissue.

Proliferating cell nuclear antigen (PCNA), a nuclear non-histone antigen, is a proliferation marker that allows for the estimation of the growth fraction in a tumor (Bedavanija et al., 2003; Liu et al., 2003). Among the microenvironment components, tumor-associated macrophages (TAMs) are the major inflammatory component of the stroma in a tumor (Mantovani, 2005). Therefore, to gain an understanding of the role of visfatin in oral cancer, we also investigated the association of visfatin with PCNA and CD68 in patients with oral cancers. Plasma visfatin concentrations in patients with OSCC and oral hyperplasia and control subjects were also examined.

Materials and methods

Sample collection

The records of 23 patients (21 males; 2 females) with oral cancer and precancerous lesions were retrieved from E-Da Hospital, I-Shou University, Taiwan (2010–2012). The mean age of the patients was 51.5 years (range, 32–70 years). All patients had primary lesions and had undergone total surgical excision. The detailed clinical and TNM data of these 23 patients are shown in Table 1. Thirteen cases occurred in the buccal, 5 in the tongue, 2 in the lips, and 3 cases were found in the palate, retromolar area, and neck, respectively. Of the 19 oral cancer

Table 1 – Patients' characteristics.

Case no.	Gender	Age	Location of primary	TNM state	Visfatin immunoscores
1	Female	61	Tongue	T1N0M0	+++
2	Male	46	Neck	T1N0M0	+++
3	Male	51	Buccal	T2N0M0	++
4	Male	40	Buccal	T2N0M0	++++
5	Male	44	Retromolar and gum	T4N0M0	++++
6	Male	39	Tongue	T1N0M0	+++
7	Male	63	Buccal	T4N0M0	+++
8	Male	52	Tongue	T2N0M0	++++
9	Male	45	Buccal	T4N2bM1	++++
10	Male	59	Buccal	Epithelial hyperplasia	++++
11	Male	52	Buccal	T2N0M0	++++
12	Female	58	Tongue	Verrucous hyperplasia	++++
13	Male	32	Buccal	T4N0M0	++++
14	Male	70	Lip	T2N0M0	++
15	Male	46	Buccal	T2N1M0	+++
16	Male	67	Buccal	Squamous papilloma	++++
17	Male	58	Lip	T3N2bM0	+++
18	Male	59	Tongue	T4N0M0	+++
19	Male	44	Buccal	T2N0M0	++++
20	Male	68	Palate	T2N0M0	++++
21	Male	53	Buccal	T4N0M0	+++
22	Male	53	Buccal and gum	T4N0M0	+++
23	Male	60	Buccal	Verrucous hyperplasia	++++

+, score 0–2; ++, score 3–5; +++, score 6–8; ++++, score 9–12.