RESEARCH ARTICLE

Expression of Bone Morphogenetic Protein-2 and Histological Differentiation of Oral Squamous Cell Carcinomas

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Abstract

Background and Objective : Bone morphogenic protein-2 (BMP-2) plays an essential role in mesenchymal cell differentiation into osteoblasts، through many intracellular pathways which may also be active in tumors. Invasive oral squamous cell carcinomas account for more than 90% of head and neck malignancies in many cancer registries. They are classified into three types according to epithelial cell differentiation. The present study aimed to identify any relation between BMP-2 expression and tumor histology. **Materials and methods:** BMP-2 expression was compared immunohistochemically among 30 cases (19 male and 11 female, mean age 48 years) of oral squamous cell carcinoma, Division was into 3 groups (each containing 10 cases) according to the histological grade. **Results:** No significant correlation between BMP-2 expression and histological grade was observed. Changes in localization and cytoplasmic staining were also not apparent. **Conclusion:** From the results of this study BMP-2 does not appear to have any application as a prognostic marker for oral squamous cell carcinomas.

Keywords: BMP-2- oral squamous cell carcinoma- histologic differentiation

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Introduction

Oral squamous cell carcinoma (OSCC) is an escalating pathology around the world. It is the third most common malignancy in south central Asia, it composes about 95% of oral cancers in India - because of the rampant tobacco and areca nut habits, leading to oral cancer being an important reason for mortality. More than 80 000 new cases are diagnosed every year, with nearly three-fourth of the patients presenting with the advanced stage of the disease (Scully and Bagan, 2009). Despite extensive research undertaken on its pathogenesis and management, the 5-year survival rate of these patients has not improved and remains less than 50% (Angadi et al., 2016).

OSCC originated from the oral keratinocyte , and as any cancer, is caused by DNA mutation, often spontaneous but increased by exposure to any kind of mutagens – chemical, physical or microbial. The various changes in the DNA can progress from a normal keratinocyte to a potentially malignant keratinocyte which able to proliferate in a less-controlled fashion than normal (Feller et al., 2013).

The oral epithelium is in a constant process of turnover, and Oral cancer refers to cancer occurring between the vermilion border of the lips and the junction of the hard and soft palates or the posterior one third of the tongue. As with most head and neck sites, squamous cell carcinoma is the most common oral cancer. This type of malignancies can arise in several places, but it is often preventable, and if diagnosed early is usually curable (Zaid et al., 2016a).

Currently, two systems are used to histologically classify tumor lesions; the International Histological Classification of Tumors and the pattern of the TIF (Rivera et al., 2011). The initial classification of lesions which is considered in the current study is based on the degree of tumor differentiation (well, moderately and undifferentiated), which is essential to evaluate the tumor's growth rate and ability to metastasize (Rivera and Venegas, 2014)

Bone morphogenetic proteins (BMPs) are a protein family secreted to the extracellular environment as a mechanism of intercellular communication and work as ligands of specific receptors that are on target cells. BMPs have recognized roles in bone formation during tissues development. These proteins , especially rh-BMP2, promote the healing of critical-size bone defects, which makes them useful in orthopedic , maxillofacial research as well as in the field of tissue engineering and regeneration of bone prior to dental implants placement. (Zaid et al., 2016 b).

Extensive studies in cultured cells, knockout mice, and humans with mutations in BMP related genes have demonstrated that the BMP pathway is indispensable for embryonic patterning as well as in neural, heart, and cartilage development (Chen et al., 2004) .

All BMPs ligands bind to two types of receptors (type I and type II), which are two transmembrane serine/threonine kinases. These receptors, normally, are separated in the plasma membrane, but when the ligand existed in the environment both receptors are associated together, working the molecule ligand as a bridge between the two

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receptors (Blanco Calvo et al., 2009).

The aim of the present study was to establish the expression of BMP2 in the histopathological degrees of oral squamous cell carcinomas.

Materials and Methods

This laboratory-based study involved the use of buffered formalin-fixed, paraffin-embedded tissues of histopathologically diagnosed cases of OSCC. A total of 30 cases were evaluated immune-histochemically for BMP2 expression , these include 10 cases of well differentiated (WDSCC), 10 cases of moderately differentiated (MDSCC) and 10 cases of poorly differentiated (PDSCC) squamous cell carcinoma.

The diagnosis was confirmed by two oral pathologists using sections stained with hematoxylin and eosin.

Immunohistochemistry

Two or three serial sections 4µm thick were prepared and placed on silanized slides. The sections were deparaffinized and rehydrated through xylene and descending grades of alcohol. Antigen retrieval was carried out in a pressure cooker in 10 mM citrate buffer (pH 6.0) for 2 to 5 min. The sections were then incubated after covering them with 3% hydrogen peroxide for 15min to block any endogenous peroxidase activity, and then slides incubated with primary Anti-BMP2 antibody polyclonal antibody (Abcam , USA) for 4h at room temperature using an optimal dilution of 1:50,

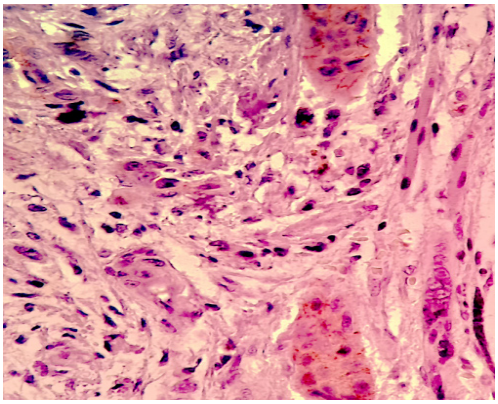

Figure 1. Expression of BMP2 in MDSCC

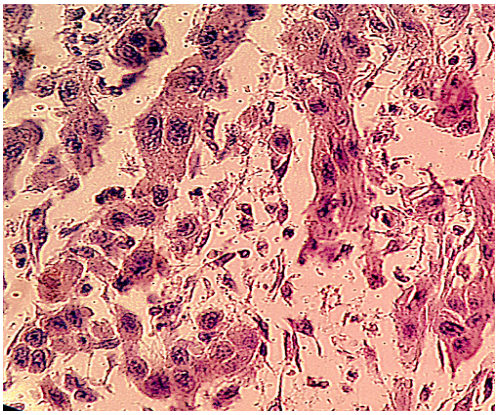

Figure 2. Expression of BMP2 in PDSCC

Table 1 . The Percentages of Staind Cells with BMP2 in the Study Sample

histological degree of OSCC	Mean	Standard deviation	Lowest value	highest value
WDSCC	8.6%	1.6%	7.9%	9.8%
MDSCC	9.8%	1.5%	8.1%	9.5%
PDSCC	9.5%	1.6%	8.2%	9.8%
Total	9.3%	1.5%	7.9%	9.8%

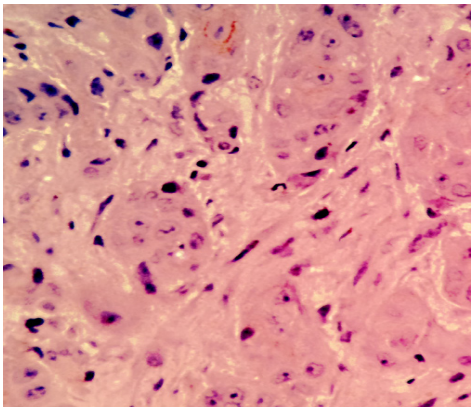

Figure 3. Expression of BMP2 in WDSCC

After further incubation with the secondary antibody (45 min) and streptavidin peroxidase (30 min), visualization was performed using freshly prepared diaminobenzidine (DAB) chromogen for 10 min. The slides were finally counterstained with Harris hematoxylin.

Five of the most invasive tumoral islands were captured and the whole epithelial cells in these islands were counted and examined. One-way Anova and Tukey's test were used for comparison and correlation between the different grades of OSCC.

Results

BMP2 was expressed in 9.3% of the cells (8.6% of WDOSCC, 9.8% of MDOSCC and 9.5% of PDOSCC) (Table 1) and (Figures 1, 2, 3) , there was no significant correlation ($p>0.05$) between BMP2 expression and the histological degrees.

Discussion

BMPs were originally isolated from the bone and are capable of inducing new bone formation at ectopic sites in vivo. BMP2- and BMP7-containing osteogenic implants have so far been used in over one million patients worldwide for the treatment of long bone nonunion, spinal fusions, and acute fractures (Wagner et al., 2010). Apart from their recognized role in bone regeneration, BMPs have been used systemically to improve skeletal volume, kidney regeneration, and glucose and iron metabolism, so it is very important to analyze these growth factors side effects especially with cancer patients.

Oral cancer is the sixth most common cancer worldwide, more than 90% of all oral cancers are squamous cell carcinomas (SCC), The effects of BMP-2 on the malignant cells still controversial and are perhaps

contingent upon the tissues and environment where they are expressed (Suzawa et al., 1999). Many researchers investigated the link between BMPs and cancer and pointed that several types of BMPs (such as BMP-2, BMP-4,BMP-6 and BMP-7) are implicated in many types of cancer tissues and also many studies reported their Dysregulation. (Mancino et al., 2008; Zaid et al., 2016 a).

In the current study no relation found between the histological differentiation of oral squamous cell carcinoma and the expression of the immune-histochemical marker of BMP2 , indicating that it cannot be used as a reliable prognostic marker in this type of malignancies . and make maxillofacial surgeons more ensure when using BMP for reconstructing bone defects after resecting advanced OSCC from the oral cavity.

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