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Original Article

The expression of transglutaminase 2 (TG-2) in oral squamous cell carcinoma and its clinical significance

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Abstract

Background: Glutamine has a very important role in the human body, including pH balance in an acidic environment, as well as supporting the TCA cycle in cancer cell growth. However, the expression of transglutaminase-2 (TG-2) in oral cancer growth related to renal function is unknown. Here we examined TG-2 and its expression as a prognostic tool.

Methods: Fifty-six oral squamous cell carcinoma (OSCC) tissues were collected with the inclusion of tumor in any region of oral area, and patients with creatinine (Cr) and blood urea nitrogen (BUN) results. The tissues were stained using immunohistochemistry (IHC) with a TG-2 antibody [N3C3], then observed under the microscope. The staining were calculated using Adobe Photoshop CS software and statistical analyses using SPSS ver. 21.

Results: We found that TG-2 expression showed a significant difference in the expression levels between tumor and the adjacent groups without disease-free survival, disease-specific survival, and recurrence between, with $p < 0.05$. The average staining intensity with 25th percentile of TG-2 becomes a vital score for the diagnosis. Furthermore, our study demonstrates a good prognosis outcome if the intensity score showed a difference in TG-2 expression between the adjacent and tumor tissue.

Conclusion: To our knowledge, this is the first clinical study on TG-2 expression in OSCC, and it demonstrates that TG-2 can serve as a predictor of tumorigenesis and prognosis outcome.

Keywords: Glutaminase; OSCC; Renal

1. Introduction

Oral cancer is a neoplastic lesion of the head and neck, and is one of the most common cancers in men. Each year, there are 6 million deaths worldwide due to oral cancer[1] and approximately 74% of oral cancer cases are due to the use of tobacco and alcohol. As a result, cessation of tobacco and smoking is the primary prevention method, followed by early detection of precancerous and cancerous lesions. More recently, 22 molecular biomarkers were identified that can be used as therapeutic and diagnostic tools to predict prognosis and survival in patients with oral squamous cell carcinoma (OSCC). Detecting the protein expression levels of such biomarkers using immunohistochemistry (IHC) is a well-recognized tool for identifying and providing clinical information on tumor specimens.[2,3] As a result, in this study we used IHC to detect transglutaminase 2 (TG-2) expression

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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levels in tumor tissues and its overexpression has been reported in several types of human cancer.[4]

Most of the energy in a normal cellular process comes from glucose through oxidative phosphorylation (OXPHOS). Glucose is converted to pyruvate by pyruvate dehydrogenase in the cytosol, and then enters the tricarboxylic acid (TCA) cycle, which provides acetyl Co-A to the mitochondria for rapid cell division. In tumor cells, the metabolic pathway changes and is reprogrammed. Specifically, instead of entering the TCA cycle, glucose is consumed *via* aerobic glycolysis, which is faster than OXPHOS, and results in produce more lactate. This was observed by Otto Warburg as a mitochondria defect in tumor cells. In order to achieve the energy, tumor cells need higher uptake of glucose to support their biosynthesis and redox.[5]

In addition to glucose, glutamine is another essential nutrient and an abundant amino acid for growing tumor cells. Under low glucose, glutamine serves as a metabolic intermediate that becomes converted to glutamate *via* the mitochondria enzyme glutaminase (GLS) in order to supply carbon to the TCA cycle for cell survival. Glutamine becomes a major source of energy for feeding the net production of oxaloacetate to produce acetyl Co-A *via* reductive carboxylation through alpha-ketoglutarate (AKG) metabolism, electron transport chain, OXPHOS, pre-cursor for biosynthesis of glutathione, nucleic acids, and certain amino acids.[6,7] While the precise role of glutamine in tumor cells is not completely understood, the genetic background and microenvironmental factors are believed to play an important role.[6,8,9]

In the normal human body, glutamine levels are approximately 70 g/d. The kidneys consume glutamine as an important donor to produce NH_3 by the action of phosphate-dependent glutamine (GLS1), and only 10% is metabolized by membrane-bound gamma glutamyl transferase in the lumen of the collecting tubule. This results in H^+ to form NH_4^+, which is then excreted in the urine. Approximately 70% of glutamate is then released back into the renal vein, and causes H^+ from carbonic acid to dissociate and form bicarbonate (HCO_3^-) and H^+. This HCO_3^- enters the circulation for pH regulation in the plasma. To fight the acidic stress, the production of ammonia by GLS1 is an important mechanism for neutralizing the pH environment caused by the toxic buildup of protons, and plays a vital role in cancer growth.[10,11] Conversely, glutamine also participates in gluconeogenesis from glutamate that is converted *via* the formation of 2-oxoglutarate by malate and oxaloacetate, or to phosphoenolpyruvate (or directly from malate to pyruvate). It also increases the formation of AKG to the TCA cycle, and for hepatic ureagenesis. The functions of the kidneys are for the maintenance of acid levels for processes like renal gluconeogenesis, the excretion of waste products, and the regulation of hematopoiesis.[6,12−14]

Given glutamine's important role in human physiology and its role in supporting the TCA cycle for cancer cell growth, it represents a potential early diagnostic and intervention tool. We hypothesized that glutamine would display higher expression in the progressing tumor, and would result in pH changes in the tumor microenvironment. To explore this notion, in this study we focused on the aspect of glutaminase in converting glutamine to glutamate for entry into the TCA cycle.

2. Methods

2.1. Ethic statement

The clinical study was reviewed by the Institutional Review Board (IRB) with approval number 2016-02-005BC at the Taipei Veteran General Hospital, and informed consent from patients was signed.

2.2. Tissue samples

The OSCC tissue samples were collected under surgical operation, and fixed in formalin. The samples included in the research are from patients with any region of tumor in oral area, without age limitation. Patients with creatinine (Cr) and blood urea nitrogen (BUN) results were observed for renal function. Samples were eliminated when diagnosed as renal abnormalities or failure. No data for renal electrolytes, or both Cr (0.7−1.4 mg/dL) and BUN (6−20 mg/dL) levels, were out of the normal range. Moreover, samples were not included if tissue loss under microscope observation was observed to be more than 50% after the IHC procedure. Only fifty-six patients met the requirements.

2.3. Immunohistochemistry

A standard IHC protocol was followed to stain the normal and tumor tissue samples. The normal and tumor tissue sections were deparaffinized with Xylene I and II, before treatment with 100% alcohol and double-distilled water (ddH_2O). During antigen retrieval, the slides were heated in a solution consisting of 47.5 mL ddH_2O and 2.5 mL Trilogy for 30 min at 75−100°C. Then tissue sections were washed in Tris-buffered saline and Tween-20 (TBST), and then repeated in 3% H_2O_2 for 20 min, before a final wash in Ultra V block for 5 min. Primary antibody (transglutaminase 2) was applied (1:200 in dilution buffer), and the slide was incubated overnight at 4°C in a humid chamber. The slides were washed in TBST, and biotinylated secondary antibody was applied before continuing with streptavidin-peroxidase. AEC chromogen was washed with ddH_2O, and the slides were dipped in hematoxylin solution for 10 s and carefully washed with tap water. The slides were then mounted with mounting medium (Dako Glycergel) and covered with glass cover slips.

2.4. Image acquisition

The slides were observed under a Zeiss Germany Axioskop 50 microscope with filter set 02 (G 365; FT 395; LP 420) and 5× magnification. The microscope was connected to a higher-performance camera (Evolution VF Cooled Color). The CCD sensor gives a resolution of 1.4 million pixels in a 12-bit

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