Oral Science International xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Oral Science International

journal homepage: www.elsevier.com/locate/osi

Review

Proteomics pattern associated with gingival oral squamous cell carcinoma and epulis: A case analysis

Francesco Papa[a], Rosa Anna Siciliano[b], Francesco Inchingolo[c], Maria Fiorella Mazzeo[b], Salvatore Scacco[a], Rosa Lippolis[d,*]

[a] Department of Basic Medical Sciences, Neurosciences and Sense Organs, University Aldo Moro, Bari, Italy
[b] Institute of Food Sciences, National Research Council (CNR), Avellino, Italy
[c] Department of Interdisciplinary Medicine, University Aldo Moro, Italy
[d] Institute of Biomembranes and Bioenergetics, Italian National Research Council (CNR), Bari, Italy

ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
24 November 2017
Accepted 5 December 2017
Available online xxx

Keywords:
Biomarkers
Proteomic analysis
Oral squamous cell carcinoma

ABSTRACT

Objectives: Oral squamous cell carcinoma (OSCC) is the most common epithelial malignant neoplasm affecting the oral cavity. OSCC can mimic oral lesions of inflammatory origin with benign features, often leading to delay in diagnosis and treatment. Early detection is important to greatly increase the chances of a successful treatment. The present study reports a proteomic analysis of a gingival oral squamous cell carcinoma (G-OSCC) and an epulis.
Materials and methods: Normal mucosae tissue, G-OSCC tissue, and epulis tissue as a comparative sample of benign nature were collected and immediately frozen in liquid nitrogen. Tissue-extracted proteins were separated by two-dimensional gel electrophoresis and subjected to image analysis. Proteins that showed a significant difference in the expression level in the G-OSCC tissue were identified by the nano-ESI-HPLC-MS/MS experiment and database searchi.
Results and conclusion: The proteomic analysis of G-OSCC tissue enabled the identification of proteins that are potentially related to the disease; these proteins can be considered as signature molecules for diagnostic and prognostic tumor markers.

Contents

Abbreviations: OSCC, oral squamous cell carcinomas; G-OSCC, gingival oral squamous cell carcinoma; 2-DE, two-dimensional electrophoresis; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; LC-ESI-MS/MS, liquid chromatography–electrospray ionization–tandem mass spectrometry.

* Corresponding author at: National Research Council (CNR), C/o Department of Basic Medical Sciences, Neurosciences and Sense Organs, P.zza Giulio Cesare 11, 70124 Bari, Italy.
E-mail address: r.lippolis@ibbe.cnr.it (R. Lippolis).

https://doi.org/10.1016/S1348-8643(17)30044-7
1348-8643/

1. Introduction

The majority of cancers in the oral cavity are oral squamous cell carcinomas (OSCC) [1], a multifactorial disease that arises from the stratified squamous epithelium lining the oral mucosa [2–4]. The tumor originates by morphological cell transformation, often through clinically benign precancerous lesions and develops according to their potential of neoplastic transformation [5–9]. Gingival oral squamous cell carcinoma (G-OSCC), classified as a subset of OSCC, is a relatively rare malignant carcinoma of the oral cavity, representing less than 10% of diagnosed intraoral carcinomas. The etiology of OSCC remains unknown, but it is established that the disease is associated with a variety of risk factors, in particular smoking and heavy alcohol use [10]. OSCC involves a series of mutations that result in the selective growth of mutated cells replacing normal cells in a specific region [11]. Because of its close proximity to the teeth and periodontium, G-OSCC can mimic other tooth-related lesions, especially those of inflammatory origin. Clinical presentation of G-OSCC can be quite variable, and hence, it is misdiagnosed as benign tumor or as inflammatory response. At present, diagnosis of oral cancer is mainly based on clinical oral examination. Histopathology is an adjuvant technique in identifying oral tumor or malignant transformation of oral lesions. The conventional diagnostic methods alone are, however, not sufficient to support early diagnosis of the disease and differential diagnosis with respect to benign tumors [12,13]. The majority (two-thirds) of OSCCs are diagnosed at an advanced stage [14] when prognosis is fairly poor, and the overall 5-year relative survival rate of oral and pharyngeal cancer patients is approximately 59% [15]. Delay in diagnosis can still be considered a major cause of the high morbidity and mortality of OSCC patients. Current standards-of-care (surgery and/or radiotherapy) often end-up with devastating consequences on the appearance and function of affected organs, thereby causing a marked detriment on the quality of life even in successfully treated patients. The molecular mechanism underlying the pathogenesis of OSCC is relatively poorly understood and represents a topic of significant importance. A better understanding of the molecular mechanisms involved in the pathogenesis and progression of the disease is a key to the development of more effective tools to improve early diagnosis of oral cancer, better prognosis, and quality of life of the patients. Discovering new reliable markers for OSCC and developing new diagnostic tools for its early and easy detection is thus a relevant issue in the field of oral pathology research.

Weinberger et al. analyzed molecular differences in protein expression among tumors that arise from different sites of the head and neck region, and they found considerable molecular diversity between different squamous cell carcinomas (SCCs) [16]. In the present study, a proteomic analysis was conducted to analyze the cellular protein profile of a case of G-OSCC and to identify potential signature molecules. Healthy tissue and non-neoplastic epulis tissue were taken as samples for comparison.

2. Materials and methods

2.1. Case report

A 58-year-old female patient reported at the Maxillofacial Unit of the Medical School of the University of Bari (Italy). Intraoral examination revealed the presence of reddish gingival growth at right lower first and second molars measuring approximately 0.5 cm, grade III mobility in the 47 tooth, recession of the marginal gingiva in the region, and generalized chronic periodontitis. Extraoral examination revealed a palpable, nontender, mobile, submandibular lymph node on the right side. On the basis of the above findings, the buccal growth was provisionally diagnosed as an inflammatory/reactive gingival growth and apical periodontitis. Information related to age and gender, smoking, alcohol consumption, clinical aspect of the lesions, and sites of oral involvement were collected. Histopathological analysis of biopsy specimen revealed a malignant neoplasia of epithelial origin, which is characterized by invasive proliferation of nests and cords of neoplastic epithelial cells. Based on histological grading, the tumor was categorized as a well-differentiated G-OSCC.

A case (55-year-old female) of epulis is also included. Epulis is present as a sessile formation on the upper right mucosa gingiva near the front of the mouth between the canine teeth and the first premolar. Its histology is related to fibrosing granulation tissue, firm and rubbery, and pale pink in color.

Sections of the normal buccal mucosa obtained from the two patients were used as the control tissue.

2.2. Reagents

Immobiline DryStrip (pH 3–10 and 4–7, 13 cm), DryStrip, cover fluid, immobilized pH gradient (IPG) buffer, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), bromophenol blue, agarose, acrylamide, tris-base, glycine, sodium dodecyl sulfate (SDS), N,N,N′,N′-tetramethylethylenediamine (TEMED), Coomassie Brilliant Blue R-250, dithiothreitol (DTT), iodoacetamide, acetic acid, and trifluoroacetic acid were purchased from GE Healthcare (Uppsala, Sweden). Sequencing grade-modified trypsin was purchased from Promega (Madison, WI, USA). The remaining chemicals were of analytical grade. All buffers were prepared with Milli-Q water.

2.3. Tissue collection

Normal mucosae tissue, G-OSCC tissue, and epulis tissue were collected with informed consent from the patients at the Maxillofacial Unit of the Medical School of the University of Bari. Inflammatory tissues were sampled from the central part of the inflammatory zone. Cancer samples were obtained from the "core" part of the tumor to avoid the adjacent noncancerous tissue by a standard mapping biopsy strategy. Small fractions of mucosal tissues were carefully removed from the pathological samples. For normal tissue, samples of surface epithelium were obtained selectively by dissection with special care for minimal contamination of nonepithelial cells. All the samples were immediately frozen in liquid nitrogen and stored in a deep freezer (−80 °C) for proteomic analyses. The study was approved by the university ethics committee.

2.4. Sample preparation for proteomic analysis

Proteins were extracted following the protocol reported by Zhang [17]. Briefly, tissue samples of normal, non-neoplastic, and malignant tissues (∼200 mg) were ground in small pieces in a mortar with liquid nitrogen, collected in tubes, and homogenized in 2 mL fresh lysis buffer composed of 7 M urea, 2 M thiourea, 40 mM Tris, 4% (w/v) CHAPS, 100 mM DTT, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), 0.5 mM ethylenediaminetetraacetic acid (EDTA), 1% TritonX-100, and 5% IPG buffer. Tissue lysate was further disrupted with an ultrasonic homogenizer and centrifuged at 12,000 × *g* for 10 min at +4 °C. Supernatant was precipitated by the addition of cold acetone (dilution ratio 1:12, v/v) and incubated at −20 °C overnight. After centrifugation at 14,000 × *g* for 15 min at +4 °C, the pellet was resuspended in rehydration buffer. The concentration of the protein extracts was determined by the modified

Please cite this article in press as: Papa F, et al. Proteomics pattern associated with gingival oral squamous cell carcinoma and epulis: A case analysis. Oral Sci Int (2018), https://doi.org/10.1016/S1348-8643(17)30044-7