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The role of components of the extracellular matrix and inflammation on oral squamous cell carcinoma metastasis

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ABSTRACT

Objectives: Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions, and is identified as the most frequently occurring malignant tumour of oral structures. We aimed to investigate the genes and pathways related with metastasis on Turkish OSCC patients.

Materials and methods: We performed whole genome expression profiling array on an Illumina platform. A total of 24 samples with 12 OSCC and 12-paired controls that had no tumour were included in the study. Hierarchic clustering and heat map were used for data visualisation and *p*-values assessed to identify differentially expressed genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Systems (IPA) analysis were performed to consider biologic meaning of differential expression of the genes between tumour and control groups.

Results: We identified 790 probe sets, corresponding to 648 genes that were effective in separating invasive and metastatic OSCC. Consequently, we found statistically relevant expression results on extracellular matrix members on MMPs such as MMP3, MMP10, MMP1 and MMP9; on laminin such as LAMC2, LAMA3 and LAMB3; several genes in the collagen family; and also on chemokines from the inflammation process.

Conclusion: Statistically relevant expression changes for MMPs, laminins, collagens, and chemokines, which are components of the extracellular matrix and inflammation process, may be considered as a molecular biomarker for early prediction. Further studies are necessary to determine and understand the molecular mechanisms that underlie OSCC metastasis.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common type of cancer with increasing rates worldwide.[1] OSCC accounts for about 90% of oral cancers and can occur at different anatomic sites of the oral cavity such as the tongue, oropharynx, lip, mouth floor, gingiva, hard palate, and buccal mucosa.[2–5] Tobacco smoking and alcohol exposure are accepted as the major risk factors for transformation through oral epithelia to cancer.[6] Despite the significant improvements in surgical techniques and chemotherapy, the five-year survival rate for OSCC patients is still approximately 30–50% and has not changed over the past decades.[7–10] OSCC has become more important not only because the survival rate is about 30–50% but also diagnosis is one of the most difficult cancer types in early stage.

Owing to the relationship between tumour development and progression with malignant cells, it is well known that the extracellular matrix (ECM), including its components, has some straightforward roles.[11,12] It is important to protect the ECM because it has an impact on a series of processes in important aspects of metabolism such as cell adhesion, migration, proliferation, differentiation, and gene expression. The structure of extracellular matrix is protected by some proteases that include matrix metalloproteinases (MMPs), laminine, members of the collagen family, and also chemokines, a sub-family of inflammation regulators, the latter being the focus of our study.[13,14] It has been reported that tumour invasion and metastases are related to degradation of the ECM in several cancer types, including OSCC.[15–19] Even though MMPs are also known for their capability of regulating apoptosis, bone remodelling and angiogenesis, they have a role in highly complex processes that is not yet clear.[20,21] Collagen, which provides structural support, is the other main component of the ECM. In some pathological conditions like cancer, collagen activity becomes abnormal thus affecting its role in the ECM.[22] Laminin is an extracellular glycoproteins that is considered as a molecular marker for detecting the basement membrane during tumorigenesis due to degradation of basement membrane that occurs in most tumours.[23–25] Moreover, inflammatory processes are also reported to be related with several types of cancer.[26] Chemokines are a member of the inflammatory-regulator family that has important roles in inflammation processes, angiogenesis, and metastasis.[27,28]

Because of the relationship between the ECM and OSCC, molecular-based studies are necessary to find multiple markers. In this respect, we aimed to simultaneously show metastatic genes and pathways for components of the ECM related with metastasis: MMPs, laminins, collagens and chemokines, in Turkish patients with OSCC. We believe that the data from this important study might ultimately lead to future research and would be helpful to find new biomarkers for early prediction of OSCC using components of the extracellular matrix.

2. Materials and methods

2.1. Tissue collection

Samples of squamous cell carcinoma were collected through the Department of Otolaryngology and the Department of Oral and Maxillofacial Surgery of Istanbul University after obtaining written informed consent from the participants and approval of Istanbul University's Ethics Committee. Tumour samples were obtained from patients with OSCC at the time of resection or biopsy prior to chemo/radiation therapy. Paired control nontumor tissues from patients were collected from the clinically unaffected side and were histologically normal. The patients and controls were matched for smoking status. Tumours were pathologically staged according to the guidelines issued by the American Joint Committee on Cancer. Tissue samples were soaked in RNAlater™ (Ambion, Austin, TX) immediately after surgical removal and transferred to the Department of Molecular Medicine of Istanbul University for long-term storage at −80 °C prior to use.

2.2. RNA extraction and target sample generation

Tissue samples were homogenised and total RNA was extracted using High Pure Tissue RNA Isolation Kit (Roche, Germany) according to the manufacturer's instructions. Quality and quantity of RNA was measured by using Nanodrop (Thermo Scientific, USA). RNA integrity was evaluated by microfluidics analysis using the Agilent® 2100 bioanalyzer (Santaclara, CA) and an RNA LabChip® Kit. The Illumina® TotalPrepTM RNA Amplification Kit was used for generating biotinylated, amplified RNA for hybridisation with Illumina Sentrix®. The procedure consisted of reverse transcription with an oligo(dT) primer bearing a T7 promoter using ArrayScript,™ a reverse transcriptase (RT) engineered to produce higher yields of first strand cDNA than wild-type enzymes. The cDNA then underwent second strand synthesis and cleanup to become a template for in vitro transcription with T7 RNA Polymerase. To maximise cRNA yield, Ambion® MEGAscript® (Invitrogen, Life Technologies) in vitro transcription (IVT) technology along with biotin-UTP (provided in the kit) was used to generate hundreds to thousands of biotinylated, antisense RNA copies of each mRNA in a sample.

2.3. Array hybridisation

cRNA (500 ng) was fragmented and added to a hybridisation mixture. Expression profiles were created using Illumina Human HT-12V4 microchip, targeting more than 47,000 probes derived from the National Center for Biotechnology Information Reference Sequence (NCBI) RefSeq Release 38 (November 7, 2009) and other sources. Hybridisation procedures were performed using the procedures described by Illumina. Arrays were scanned on iScan System (Illumina).