GENE EXPRESSION PATTERNS OF CHEEK, GUM AND TONGUE
SQUAMOUS CELL CARCINOMA

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ABSTRACT

Clinical evidence suggested that biological behavior as well as response to treatment is different in OSCC arising from the different anatomical locations of the mouth and could be influenced by the activation or/and inactivation of different genes and pathways. **Objective:** Gene expression analysis of OSCC from different sites of the oral cavity was conducted to determine if there were significant differences in the expression pattern that could be associated with clinical observations. **Materials and Methods:** Formalin-fixed paraffin-embedded tissues from OSCC from cheek, gum, tongue and non-cancerous oral mucosal from the matching sites were used in microarray experiments (DASL, Illumina) to determine the gene expression patterns. Microarray data were analysed using Genespring to identify differentially expressed genes and these changes were validated using quantitative polymerase chain reaction and immunohistochemistry. The role of specific genes in driving OSCC were determined using cell lines genetically modified to exogenously express these genes. **Results:** This study demonstrated that FFPE tissues can be used for microarray experiments. Differentially expressed genes in OSCC were identified and their expressions were validated in independent samples. Principal Component Analysis demonstrated that different sites of OSCC have distinct gene expression profiles. Genes that were commonly altered in all sites and those that were distinct to a particular site were identified. Focusing on a gene *FOLR1* that was found to be enriched in OSCC of the tongue, exogenous expression of this gene was shown to promote migration and invasion. **Conclusion:** This study suggests that the genetic progression of OSCC in the different sites is distinct, thus cautioning the generalization of OSCC when identifying biomarkers for diagnosis, prognosis and therapy. Furthermore, specific genes may confer different cancer traits that may explain the clinical differences seen in the different sites of OSCC.
ABSTRAK

Bukti klinikal mencadangkan tingkah laku biologi serta respons kepada rawatan berbeza di kalangan OSCC dari lokasi berbeza di mulut. Ia berkemungkinan dipengaruhi oleh pengaktifan atau/dan penyahaktifan gen serta tapak jalan gen yang berbeza. **Objektif:** Analisis ekspresi gen OSCC dari lokasi berbeza di mulut dijalankan untuk menentukan jika ada perbezaan signifikan dalam pola ekpresi berkaitan dengan pemerhatian klinikal.

**Bahan dan Kaedah:** “Formalin-fixed paraffin-embedded tissue” dari OSCC daripada bahagian pipi, gusi, lidah serta juga tisu bukan-kanser oral mukosa dari bahagian yang sama digunakan di dalam eksperimen mikroatur gen (DASL, Illumina) untuk menentukan pola ekpresinya. Gen-gen yang mengalami perubahan ekpresi yang signifikan pada OSCC dikenalpasti dengan Genespring dan disahkan melalui tindak balas rantai polimerase kuantitatif dan immunohistokimia. Peranan gen yang spesifik dalam memacu OSCC ditentukan dengan mengubahsuai sel OSCC secara genetik untuk meluahkan gen secara eksogenous. **Keputusan:** Kajian ini menunjukkan yang tisu FFPE boleh digunakan untuk eksperimen mikroatur gen. Gen yang mengalami perubahan pengekpresan yang signifikan di dalam OSCC dikenalpasti dan disahkan menggunakan sampel OSCC yang berlainan. “Principal Component Analysis” menunjukkan OSCC dari lokasi berbeza di mulut mempunyai pola pengekpresan gen yang berbeza. Ekspresi gen yang berubah dalam ketiga-tiga lokasi dan yang hanya berubah di lokasi tertentu dikenalpasti. Bertumpu kepada gen *FOLR1* yang diperkayai dalam OSCC lidah, luahan eksogenous menunjukkan yang ianya menggalakkan ciri-ciri migrasi dan invasi.

**Kesimpulan:** Kajian ini menunjukkan yang perkembangan genetik OSCC berbeza mengikut lokasi berlainan di mulut. Oleh itu, perhatian perlu diberi kepada faktor ini apabila menyamaratakan OSCC dalam pencarian biopenanda diagnosis, prognosis dan terapi. Gen tertentu juga boleh memberikan ciri kanser yang berlainan, seterusnya menjelaskan perbezaan klinikal yang dilihat pada OSCC dari lokasi yang berbeza.
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ABBREVIATIONS

ALL       Acute Lymphocytic Leukemia
AM        Gum
AML       Acute Myelogenous Leukemia
APS       Ammonium Persulphate
ASR       Age Standardised Ratio
B         Cheek
BSA       Bovine Serum Albumin
cDNA      Complementary Deoxyribonucleic Acid
CO₂       Carbon Dioxide
Ct        Cycle Threshold
DAB       Diaminobenzidine
DASL      cDNA Mediated Annealing, Selection, Extension And Ligation Assay
DAVID     Database for Annotation, Visualization And Integrated Discovery
dH₂O      Distilled Water
DMEM:F12   Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
DMSO      Dimethyl Sulfoxide
DNA       Deoxyribonucleic Acid
dNTP      Deoxyribonucleotide Triphosphate
EDTA      Ethylenediaminetetraacetic Acid
FBS       Fetal Bovine Serum
FC        Fold Change
FDR       False Discovery Rate
FFPE      Formalin Fixed Paraffin Embedded
FOM       OSCC originating from Floor of Mouth
G         OSCC Originating from Gum
g        Relative Centrifugal Force
g/L      Gram per Liter
GEO       Gene Expression Omnibus
gm        Gram
GO        Gene Ontology
HC        Hierarchical Clustering
HPF       High Power Field
HPV       Human Papilloma Virus
I8        Gingival Tissues Obtained During the Surgical Removal of Impacted Wisdom Tooth
IARC      International Agency for Research on Cancer
ICD       International Classification Of Disease
IHC       Immunohistochemistry
kD        Kilodalton
KEGG Pathways Kyoto Encyclopedia of Genes and Genomes
M         Molar
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<td>mg</td>
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<td>ml/100 ml</td>
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