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Original Article

Inflammation related genes are upregulated in surgical margins of advanced stage oral squamous cell carcinoma

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ABSTRACT

Aims: Advanced stage of oral squamous cell carcinoma (OSCC) exhibits different properties compared with the early stage for example an invasion ability. The present study investigated a differential gene expression of surgical margin between advanced and early stage of OSCC.
Methods: Gene Expression Omnibus dataset (GSE31056) was downloaded and re-analyzed. Surgical margin samples were categorized into 2 groups; early stage and late stage. Differential gene expression analysis was performed. Dysregulated genes were further analyzed for gene ontology, enriched pathway, and disease association using a network-based analysis tools.
Results: Eighty-five dysregulated genes were identified in margin of late stage OSCC. Metabolic process and biological regulation were the main gene ontology of dysregulated genes. Genes involved in Jak-STAT signaling pathway were upregulated in late stage of surgical margin samples. In addition, seven upregulated genes in late stage group, namely *CEBPB*, *S1PR1*, *IL6*, *CEBPD*, *CHI3L1*, *PTX3*, and *SOCS3*, were categorized in acute phase reaction and inflammation categories of disease association analysis.
Conclusion: The differential expressed genes in surgical margin of late stage OSCC could be further employed to understand cancer's behavior and to identify target pathway to prevent OSCC invasion.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the common oral cancers.[1,2] Prevalence of OSCC is range from 60% to 100% of all oral cancers and is high in Asian country due to predisposing risk factors for example betel nut chewing.[1–3] A lymph node metastasis, tumor volume, and tumor invasiveness are factors related to poor prognosis.[4,5] Various pathways were dysregulated in OSCC compared to the normal tissues including metabolic pathway, extracellular matrix-receptor interaction, focal adhesion, cytokine-cytokine receptor interaction, and cell cycle progression.[6]

Surgical resection is one of the treatment options of OSCC. Margin assessment is indeed an essential prognostic factor. Gross inspection and frozen section evaluation have been employed to identify surgical margin.[7] Margin dysplasia is related to the lower survival rate.[8] However, studies illustrated that surgical margin of the lesion which appears normal in histological observation exhibited a genetic alteration. These molecular changes could lead to the recurrence of OSCC after resection.[9] In this respect, the positive staining of p53 was observed in basal and parabasal cells in the surgical margin.[10] Previous work also reported that the expression of *MMP1*, *COL4A1*, *P4HA2*, and *THBS2* in surgical margin was associated with OSCC recurrence.[9]

Gene expression in the margin has been shown to correlate with the aggressiveness of OSCC, for example the VEGF over-expression.[11] This gene were highly expressed in tumor margin compared with OSCC and in stage 3–4 compared with stage 1–2.[11] These results suggest that the differential expression of gene expression in the tumor margin at different cancer stage may participate in OSCC progression.[11] The present study aimed to investigate a differential gene expression of surgical margin between advanced and early stage of OSCC.

2. Methods

2.1. Dataset processing

Public available microarray expression profiling of OSCC was identified from Gene Expression Omnibus (GEO) database. GEO

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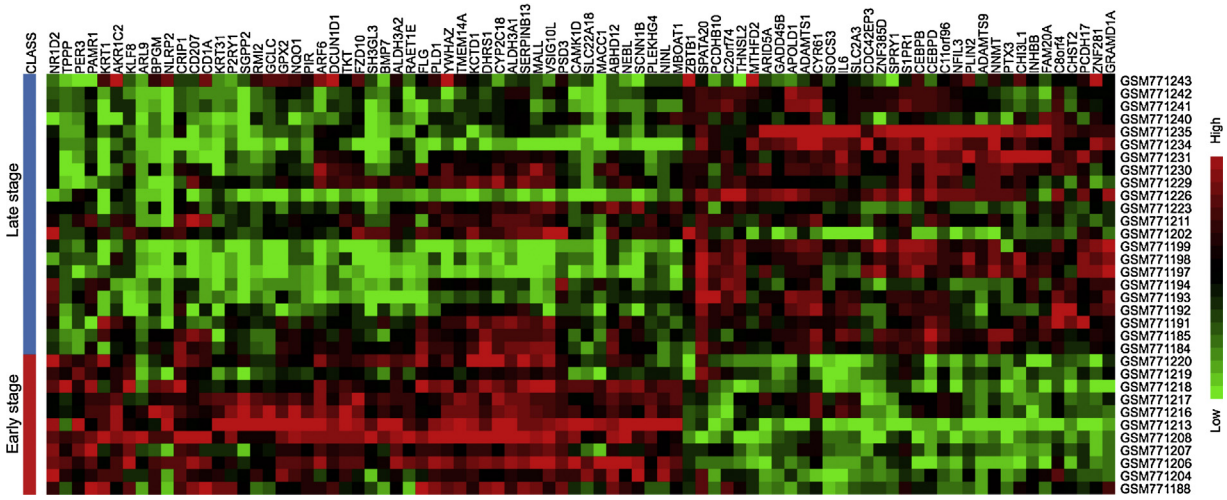

Fig. 1. Heatmap demonstrated the differentially expressed genes between surgical margins of early and late stage OSCC.

dataset, GSE31056, was downloaded.[9] Probe IDs that did not match official gene symbol or matched with multiple official gene symbol were excluded from the analysis. The included criteria for GSE samples were (1) surgical margin; (2) known disease stage. Samples from tumors and normal tissues were excluded. The included GSE samples were categorized into 2 groups. First group was the surgical margin samples of OSCC stage 1 and 2. The second group was the surgical margin samples of OSCC stage 3 and 4.

2.2. Bioinformatic analysis

Processed dataset was uploaded to NetworkAnalyst, a network-based analysis of gene expression data.[12–14] Mean intensity was calculated and variance data filtering was set at 15%. Limma statistical method was employed. Differentially expressed genes, which exhibited adjusted p-value <0.05 and log 2 fold change >1, were included for further analysis. Heatmap visualization of differentially expressed genes was performed using NetworkAnalyst. Gene ontology, KEGG enriched pathway, pathway commons, disease association analysis were performed using WebGestalt.[15,16] The analyses were performed using hypergeometric methods with significance level set at $p < 0.05$.

3. Results

Dataset contained 11 samples for group 1 (margin of early stage OSCC) and 22 samples for group 2 (margin of late stage OSCC). Total of 85 dysregulated genes were identified, composing of 34 upregulated and 51 downregulated genes in margin of late stage OSCC (Fig. 1). Top 10 differentially expressed genes were shown in Table 1. Gene ontology analysis of biological process demonstrated that majority of upregulated genes were related to metabolic process while the downregulated genes were involved in biological regulation (Fig. 2). Main dysregulated genes were categorized on membrane and protein binding group in cellular component and molecular function categories (Fig. 2). KEGG pathway enrichment analysis demonstrated that the upregulated genes were clustered in Jak-STAT signaling pathway (Table 2). Corresponding to Pathway Commons analysis, IL6-mediated signaling events and Interleukin-6 signaling categories were observed for the upregulated genes (Table 3). While the downregulated genes were clustered in metabolic pathway in both KEGG and Pathway Commons analysis. Using disease association analysis, seven upregulated genes were categorized in acute phase reaction and inflammation (Table 4). These genes were *CEBPB*, *S1PR1*, *IL6*, *CEBPD*, *CHI3L1*, *PTX3*, and *SOCS3*. Among these, the upregulation of *IL6*, *CHI3L1*, and *PTX3* was higher than 3.5 folds (Fig. 3).

4. Discussion

It should be noted that the present study identified the dysregulated genes using bioinformatics re-analysis of public available expression microarray database without the validation information of tissue expression. Hence, the interpretation and further applications should be done with caution. The validation of the dysregulated genes in the tissue sections either by polymerase chain reaction or immunocytochemistry staining is indeed necessitated. However, previous report of this dataset has been validated.[9] In addition, it has been demonstrated some dysregulated genes identified in the present study in the tissue samples. NAD(P)H dehydrogenase, quinone 1 (NQO1) mRNA expression was significantly higher in the margin of late stage OSCC than those of

Table 1
Top 10 differentially expressed genes.

Gene symbol	Gene name	Entrez ID	Log 2FC	Adjusted p value
TPPP	Tubulin polymerization promoting protein	11076	2.0765	1.40e−03
PER3	Period homolog 3 (Drosophila)	8863	1.0577	1.96e−03
KRT31	Keratin 31	3881	4.0921	2.51e−03
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	1052	−1.5553	3.59e−03
ZNF281	Zinc finger protein 281	23528	−1.0125	3.59e−03
PAMR1	Peptidase domain containing associated with muscle regeneration 1	25891	1.7659	7.32e−03
P2RY1	Purinergic receptor P2Y, G-protein coupled, 1	5028	1.7692	8.72e−03
NQO1	NAD(P)H dehydrogenase, quinone 1	1728	2.0162	1.26e−02
APOLD1	Apolipoprotein L domain containing 1	81575	−1.9391	1.26e−02
ALDH3A2	Aldehyde dehydrogenase 3 family, member A2	224	1.084	1.26e−02

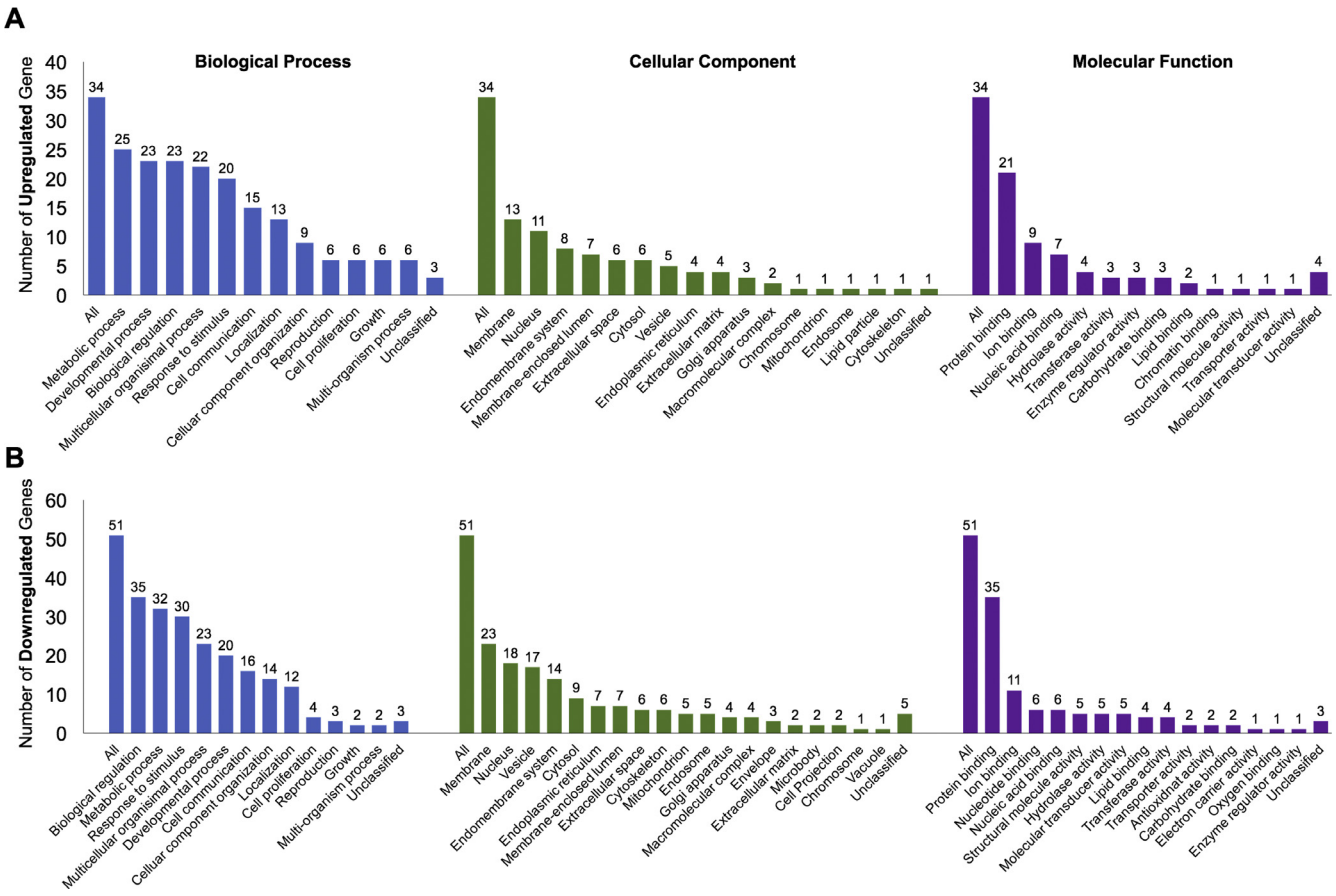

Fig. 2. Gene ontology analysis of the differentially expressed genes between surgical margins of early and late stage OSCC: (A) upregulated genes and (B) downregulated genes.

early stage samples. Correspondingly, previous work demonstrated that NQO1 was expressed in head and neck squamous cell carcinoma and their margins.17 Though, the normal margin exhibited lower NQO1 protein expression than those of cancer tissues. Further, KRT31 was upregulated in metastatic head and neck carcinoma, while less expression was noted in normal and N0/N1 lesion.18 These evidences confirmed the expression of dysregulated genes in cancer and its margin.

The present study demonstrated that surgical margin of late stage OSCC expressed higher genes in Jak-STAT signaling pathway, namely *IL6*, *SOCS3*, and *SPRY1*. Corresponding with previous study, a meta-analysis of expression profiling of OSCC showed the upregulation of Jak-STAT pathway compared to the normal tissues. 6 STAT3 dysregulation involved in carcinogenesis, treatment resistance and immune escape of head and neck squamous cell carcinoma (HNSCC).19,20 Thus, several studies targeted this pathway as a candidate treatment approach. It has been shown that Jak kinase inhibition resulted in the reduction of HNSCC cell proliferation *in vitro* and tumor growth *in vivo*.21 In addition, an active compound of *Magnolia officinalis*, Honokiol, induced OSCC

Table 2
KEGG pathway enrichment analysis.

Pathway name	Number of genes	Adjusted *p* value
Downregulated genes		
Metabolism of xenobiotics by cytochrome P450	4	0.0004
Glutathione metabolism	3	0.0018
Glycosis/gluconeogenesis	3	0.0024
Drug metabolism-cytochrome P450	3	0.0024
Endocytosis	4	0.0038
Histidine metabolism	2	0.0048
Metabolic pathways	8	0.0105
Glycerolipid metabolism	2	0.0105
Arachinodic acid metabolism	2	0.0129
Glycerophospholipid metabolism	2	0.0208
Fc gamma R-mediated phagocytosis	2	0.0255
Upregulated genes		
Jak-STAT signaling pathway	3	0.0066

Table 3
Top 10 pathway commons analysis.

Pathway name	Number of genes	Adjusted *p* value
Downregulated genes		
Glutathione conjugation	2	0.0408
Upregulated genes		
IL6-mediated signaling events	4	0.0000851
AP-1 transcription factor network	7	0.0017
Integrin-linked kinase signaling	7	0.0017
Interleukin-6 signaling	2	0.0017
Nectin adhesion pathway	8	0.0022
EGF receptor (ErbB1) signaling pathway	8	0.0022
VEGF and VEGFR signaling network	8	0.0022
PDGFR-beta signaling pathway	8	0.0022
Alpha9 beta1 integrin signaling events	8	0.0022
Arf6 trafficking events	8	0.0022

apoptosis *via* the suppression of Jak-STAT, Akt and Erk pathway.[22] Together, the overexpression of Jak-STAT signaling pathway in margin of late stage OSCC may link to the tumor aggressiveness.

IL6 acts as both pro- and anti-inflammatory cytokine. Serum and saliva IL6 protein levels were significantly increased in OSCC patients compared to the healthy control.[23,24] IL6 promoted migration of OSCC cells *in vitro*.[22] Further, IL6 expression correlated with pattern of invasion, vascular invasion and pathological nodal status.[25] IL6 overexpression led to the decrease of disease free survival rate in OSCC patients.[25] It has been shown that *SOCS3* polymorphisms was linked with the risk of head and neck squamous cell carcinoma.[26] Upregulation of *SOCS3* was observed in OSCC compared with the control.[27] *SPRY1* is a modulator of a receptor tyrosine kinase signaling. Though, role in OSCC has not yet been reported. Among these three genes, *IL6* fold change was the highest (4.32 folds). Thus, IL6 expression could be used as a marker to identify normal tumor margin.

After performing disease association analysis, the acute phase reaction and inflammation categories were identified for the upregulated genes. Genes that related to these two categories were *CEBPB*, *S1PR1*, *IL6*, *CEBPD*, *CHI3L1*, *PTX3*, and *SOCS3*. Beside the influence of *IL6* and *SOCS3* in OSCC described above, *PTX3* is another molecule which has been investigated the participation in OSCC. *PTX3* has been proposed as a maker for cancer-related inflammation. *PTX3* was induced in fibroblast after co-culture with OSCC cell line *via* IL1 signaling.[28] However, an influence of *CEBPB, S1PR1, CEBPD*, and *CHI3L1* in OSCC has not yet been identified. It has been shown that *CHI3L1* overexpression was associated with poor prognosis of thyroid carcinoma.[29] CHI3L1 may contribute to breast cancer growth and metastasis as it induced the expression of CCL2, CXCL2, and MMP9.[30] *CEBPB* and *CEBPD* were shown to be involved in the chemoresistance in other cancer cell types.[31,32] *S1PR1* expression correlated with poor prognosis in urothelial carcinoma.[33]

Inflammation induced/mediated carcinogenesis in OSCC has been widely investigated.[34] Anti-inflammatory medication are considered to be combined with conventional OSCC treatment.[34] However, the inflammation rather promotes cancer progression than induced cancer formation.[35] Corresponding with the present study, the upregulation of inflammation related genes at tumor

Table 4
Disease association analysis for upregulated genes.

Pathway name	Gene symbol	Adjusted *p* value
Acute phase reaction	*CEBPB, IL6, CEBPD, PTX3, SOCS3*	1.58e−08
Inflammation	*CEBPB, S1PR1, IL6, CEBPD, CHI3L1, PTX3, SOCS3*	5.92e−06

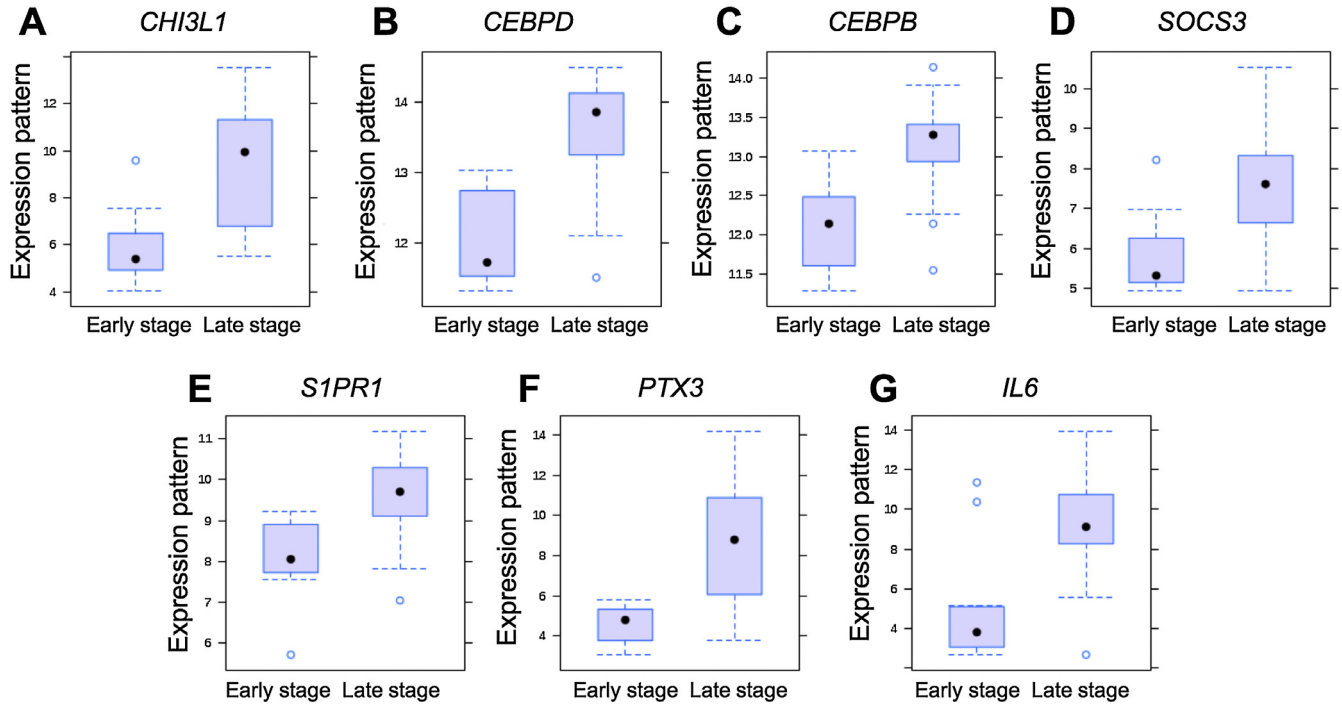

Fig. 3. Expression pattern of upregulated genes involved in acute phase reaction and inflammation categories in disease association analysis.

margin of late stage OSCC may participate to recurrence and progression of the disease.

Conflict of interest

The authors have none to declare.

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