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Dysregulation of Notch signaling related genes in oral lichen planus

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ABSTRACT

Objective: To investigate a dysregulation of Notch signaling in oral lichen planus (OLP) using public available microarray dataset.
Methods: A mRNA expression profiling dataset from Gene Expression Omnibus was downloaded. Differential gene expression between OLP and normal oral epithelium was examined using NetworkAnalyst. The dysregulated genes related to Notch signaling were identified.
Results: Thirteen genes in Notch signaling pathway were significantly differential expressed between OLP and normal epithelium. OLP samples significantly increased the mRNA levels of *HEYL*, *APH1B*, *CNTN1* and *PSEN2*. Whilst, *ITCH*, *HES1*, *TLE2*, *DLK2*, *DTX2*, *NOTCH3*, *JAG2*, *RFNG*, and *SPEN* were downregulated in OLP groups.
Conclusions: Notch signaling was dysregulated and may participate in pathophysiologic process in OLP.

1. Introduction

Oral lichen planus (OLP) is a immune-mediated disease of oral mucosa frequently found in adult female [1]. For pathophysiology, it has been shown that an activation of $CD8^+$ T cells triggers oral epithelial cell apoptosis via TNF-α or Fas–Fas ligand mechanism [1]. T cells isolated from OLP express a high level of matrix metalloproteinase leading to the destruction of basement membrane [1]. In addition, mast cells also participate in OLP pathophysiology as they secrete various pro-inflammatory cytokines initiating epithelial cell apoptosis and activating matrix metalloproteinase enzyme, resulting in the disruption of basement membrane [1].

Gene expression analysis of OLP illustrates that epithelial specific keratins, keratin 4 and 8, are downregulated in OLP compared with the normal epithelium while genes related to epithelial cell differentiation (such as filaggrin, loricrin, and repetin) are upregulated [2]. Further, OLP and genital lichen planus shared sets of common differentially expressed genes, implying the similar disease mechanism [2]. Another study demonstrated that the dysregulated genes were categorized in various functional pathways for example signal transduction, transcriptional regulation, cell adhesion, cell proliferation and apoptosis as well as inflammation and immune response [3].

Notch signaling pathway regulates epithelial cell proliferation, migration and differentiation [4,5]. Notch1 and Jagged1 are expressed in basal cell layer in human and rat esophageal epithelium [4]. Activation of Notch signaling results in the increased expression of involucrin, cytokeratin 10, and filaggrin in keratinocytes as well as the formation of stratification *in vitro*, implying the induction of epithelial cell differentiation [4]. Limited evidences are reported regarding Notch signaling in oral epithelial cells. It has been demonstrated that Notch1 expression was weakly observed in normal oral epithelium [6]. After serial *in vitro* subculture, Notch target gene, *HES1*, is upregulated [7]. Dysregulation of Notch signaling expression in oral epithelium has previously been reported in several oral epithelial diseases including oral dysplasia and oral squamous cell carcinoma [8–10]. Inhibition of Notch signaling leads to the reduction of cell proliferation in oral squamous cell carcinoma cell line [10]. However, Notch signaling regulation in OLP has not yet been reported. The present study aims to investigate the dysregulation of Notch signaling related genes in OLP.

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2. Materials and methods

Gene expression profiling in public database was searched and the dataset describing the mRNA expression profile of OLP compared with the normal epithelium was identified. The identified dataset, GSE52130, was downloaded from Gene Expression Omnibus (GEO) database [2]. Subsequently, the differential gene expression was performed using a network-based visual analytics for gene expression profiling, meta-analysis and interpretation, NetworkAnalyst [11–13]. After upload dataset, a data annotation was performed. Mean calculation was employed for gene-level summarization. Inter quartile range at 15% was used for variance data filtering and Limma algorithms was applied. Differential gene expression analysis was calculated with the selection of false discovery rate adjusted P-value < 0.05. Heatmap visualization and enriched pathway analysis were performed using add-in function of NetworkAnalyst. Notch signaling related genes were listed according to previous publication [14]. Protein–protein interaction network was analyzed and first-order network was performed using NetworkAnalyst with STRING interactome database [15] at the confidence score cutoff 900.

3. Results

3.1. Overall differential gene expression

A total of 508 genes were significantly differential expressed more than 2 folds between the normal epithelium and OLP.

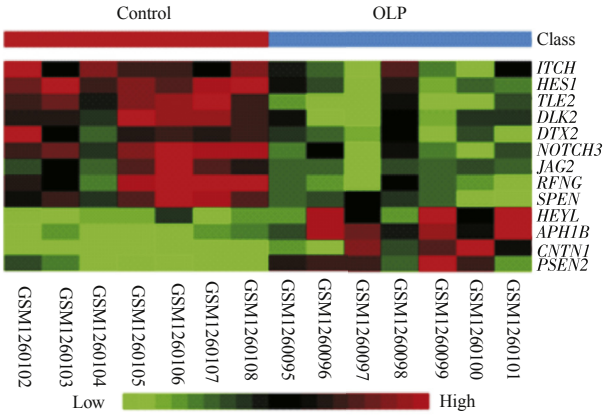

Figure 1. Heatmap illustrated the differential expression of Notch signaling component in OLP samples compared with the normal epithelium.

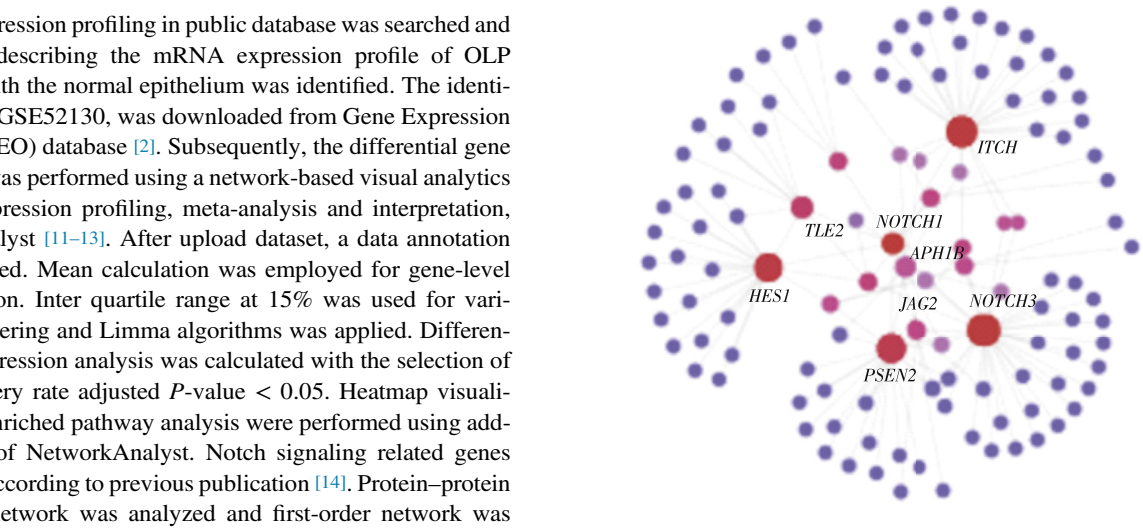

Figure 3. Protein–protein interaction network of the differential genes related to Notch signaling.

KEGG enriched pathway analysis illustrated that the dysregulated genes were categorized in 1) ECM-receptor interaction and 2) histidine metabolism pathway. Corresponding with Reactome pathway investigation, the dysregulated genes were in the following categories: 1) degradation of collagen, 2) assembly of collagen fibrils and other multimeric structures, 3) degradation of the extracellular matrix, 4) Phase 1-Functionalization of compound, 5) extracellular matrix organization, 6) collagen formation, 7) collagen biosynthesis and modifying enzymes, 8) amine oxidase reactions, and 9) crosslinking of collagen fibrils.

3.2. Notch signaling pathway dysregulation

Thirteen genes related to Notch signaling pathway were significantly different between the control and OLP samples (Figure 1). However, five genes were differentially expressed more than 2 folds change, namely *DTX2*, *HES1*, *TLE2*, *CNTN1* and *APH1B* (Figure 1B). *DTX2*, *HES1*, and *TLE2* were down-regulated while *CNTN1* and *APH1B* were upregulated in OLP samples (Figure 2). Protein–protein interaction network of the differential genes related to Notch signaling was constructed. DLK2 was not shown in the network, indicating no protein interaction (Figure 3).

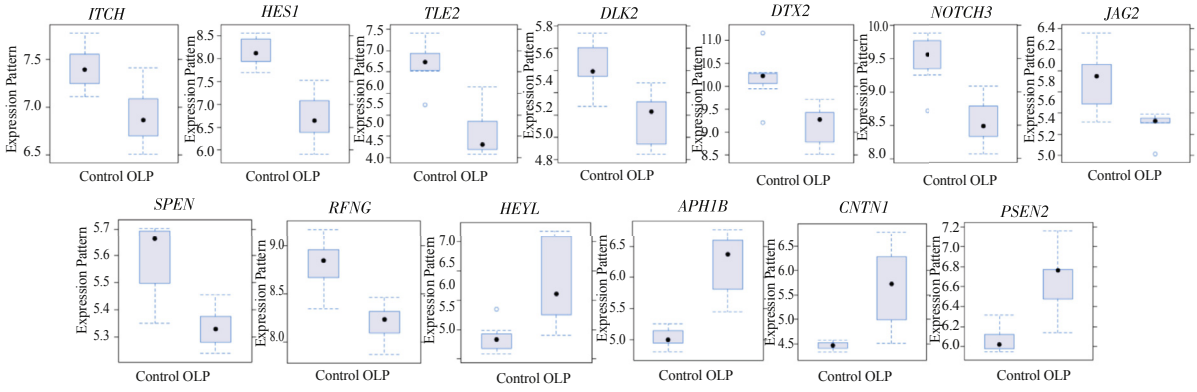

Figure 2. Graphs demonstrated expression values of differentially expressed Notch components in OLP and normal epithelium.

4. Discussion

Dysregulation of Notch signaling has been reported in several oral diseases, including oral epithelial dysplasia and oral squamous cell carcinoma. Previous report illustrated Notch signaling upregulation in oral squamous cell carcinoma compared with the control epithelium [10]. Chemical inhibition of γ-secretase preventing Notch signaling transduction led to the reduction of cancer cell proliferation [10]. Correspondingly, *HES1* expression increased in oral squamous cell carcinoma and dysplastic lesion [8]. On the contrary, Notch1 downregulation was observed in oral cancer and oral epithelial dysplasia [16]. Further, the evidences demonstrated the relationship of Notch1 mutation in oral squamous cell carcinoma patients [17]. Thus, the influence of Notch signaling on oral cancer is still controversial. Role of Notch signaling in OLP lesion has not yet been identified. The present study showed the dysregulation of Notch signaling component in OLP samples. The downregulated genes were involved in several Notch components: endosomal sorting/membrane trafficking regulators, target genes, ligand, receptor, glycosyltransferase modifiers, and nuclear effectors. While the upregulated genes were categorized in target genes, ligands, and receptor proteolysis. Thus, the current information could not conclude the direction of upregulation or reduction of Notch signaling participating in OLP. Further molecular pathophysiological study should be investigated to elucidate the role of Notch signaling in OLP.

HES1 is a common target gene in canonical Notch signaling pathway. The present study reported the downregulation of *HES1* in OLP samples compared with the normal epithelium. Corresponding with previous work, RNA sequencing analysis revealed that *HES1* expression was decreased in both OLP and oral squamous cell carcinoma samples [18], implying the participation of *HES1* in the molecular pathophysiology mechanism in OLP.

DTX2 is a member of Deltex proteins, regulating and activating Notch signaling depended on cellular context. Notch/Deltex pathway is considered as non-canonical pathway since it is not mediated by CSL pathway. Study on the function of DTX2 in mammalian cells is limited. It was previously shown that DTX2 regulated myogenic differentiation of skeletal muscle stem cells [19]. Influence of DTX2 in OLP and oral epithelial cells should be further investigated to identify the significant function in the progression of OLP.

TLE corepressors interact with various transcriptional factors and subsequently convert these transcriptional factors from activators to repressors [20]. However, role of TLE2 in oral keratinocyte as well as the pathophysiology of oral mucosal diseases has not yet been reported. It has been shown in epithelial cells that TLE expression correlated with Notch expression [21]. Correspondingly, the present study demonstrated the downregulation of *TLE2* corresponding with the decrease of *HES1* expression, confirming the association of Notch signaling upregulation and TLE expression. These evidences may imply the role of TLE in epithelial transformation in OLP patients (*i.e.* metaplasia and neoplasia).

CNTN1 is a glycosylphosphatidylinositol-anchored neuronal membrane protein, acting as cell adhesion molecule [22]. This protein could bind to Notch1 and further activate Notch signaling pathway [23]. CNTN1 was reported as the molecule regulating cancer cell metastasis [24]. CNTN1 expression correlated with the regional lymph node metastasis status in oral squamous cell carcinoma patients [25]. Consistently, upregulation of CNTN1 in esophageal squamous cell carcinoma was related to the cancer stage, lymph node metastasis and lymphatic invasion [26]. Knockdown *CNTN1* expression in oral squamous cell carcinoma resulted in the reduction of cancer cell invasion *in vitro* [25]. The present study reports the upregulation of *CNTN1* in OLP samples similar to those observed in oral squamous cell carcinoma, implying the potential involvement of CNTN1 on transformation of OLP.

APH1B is a core subunit of γ-secretase which cleaves Notch receptors at intracellular site, leading to the release of Notch intracellular domain. It has been demonstrated that the downregulation of *APH1B* expression correlated with a poor survival rate in breast cancer patients [27]. Further, an *APH1B* expression has been identified as a biomarker for risk prediction of atherosclerosis progression [28]. However, a role of APH1B in epithelial cells and its associated with oral diseases has not yet been reported. The present study demonstrated the upregulation of *APH1B* in OLP samples corresponding with the increase of *PSEN2* expression. *PSEN2* is also a component of γ-secretase enzyme and its expression significantly correlates with *APH1B* in breast cancer [27]. The combination of PSEN and APH1 subunits (PSEN1, PSEN2, APH1A, and APH1B) led to the different types of γ-secretase enzyme and exhibited preferential substrate specificity [29].

In conclusion, Notch signaling components are dysregulation in OLP lesion. Both upregulated and downregulated genes were identified. However, further investigation should be performed to clarify the role of Notch signaling in OLP pathogenesis.

Conflict of interest statement

Authors declare no conflict of interest.

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