

Role of Mutation of NOTCH1 gene in development of oral squamous cell carcinoma: a narrative review

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Abstract

Cancer of the oral cavity has numerous types and, among all, oral squamous cell carcinoma represents >90% of all cancers of the oral area. Oral squamous cell carcinoma arises from the squamous lining of the oral cavity. Across the globe, most commonly it develops in the regions of tongue followed by floor of the mouth, and lower lip. Neurogenic locus notch homolog protein 1 gene has its association with oral squamous cell carcinoma and is known to be associated with both oncogenic and tumour suppressor roles. The current narrative review comprised literature published from 2013 to 2023. It was searched on Google Scholar, PubMed and Google databases. Globally, neurogenic locus notch homolog protein 1 mutations are associated with the development of oral squamous cell carcinoma. Most of the mutations are linked to ligand bind epidermal growth factor-like repeat region of extracellular domain of neurogenic locus notch homolog protein 1. Once activated, the pathway is involved in tumour progression and metastasis. The Asians compared to Caucasians are more affected by neurogenic locus notch homolog protein 1 mutations.

Keywords: Mutation, NOTCH1, gene, Development, Oral squamous cell carcinoma.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is ranked among the commonest cancers; the sixth most common cancer out of all the cancers.¹ HNSCC is developed from the mucosal epithelial lining of oral cavity, pharynx and larynx.² Cancer of the oral cavity has several types and, among all, oral squamous cell carcinoma (OSCC) represents >90% of all cancers of the oral region.³ Oral cavity consists of two parts; oral cavity proper and vestibule. Oral cavity proper is bounded anteriorly by teeth, while posteriorly the boundary is

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provided by fauces. The vestibule is the region between cheeks and teeth along with teeth and tongue. The mucosa of the cavity is lined by stratified squamous epithelium. OSCC arises from the squamous lining of the oral cavity and can develop in various regions, including gingiva, tongue, buccal cavity, alveolar region, salivary glands, oropharynx, retro-molar space, roof and floor of the mouth.⁴

It is considered to be among the cancers which carry high mortality and morbidity.⁵ The OSCC's survival rate is considered to be poor and there is <50% chances of 5-year survival.⁶ An Iraqi study documented the tumour as being aggressive and severe in nature, and late diagnosis makes it difficult to control.⁴ The incidence of OSCC is variable across the globe. According to World Health Organisation (WHO), the incidence of OSCC is on a higher level in regions falling under its South-East Asia Regional Office (SEARO) and the European Regional Office (EURO), while the incidence is on a lower side in countries of Eastern Asia, Central America and Africa. Pakistan, India, Taiwan and Sri Lanka which are included in SEARO. The differences in the reported incidences across the world highlights the linkage of aetiological factors and genetic association with OSCC.^{7,8}

According to histological grading, majority of OSCC patients were moderately differentiated (67.7%), while poorly differentiated and well differentiated were 16.6% and 15.7%, respectively.⁹ Another study in Karachi revealed the moderately differentiated cancer (grade II) to be the commonest (59%), followed by well differentiated grade I (36%) and poorly differentiated grade III (5%). The clinical staging revealed 52% subjects involved in stage III/IV cancer.¹⁰ Yoshida et al. reported significant positive correlation of neurogenic locus notch homolog protein 1 (NOTCH1) gene expression with T-stage and clinical staging.¹¹ The aetiological factors for OSCC are variable. Yasin et al. documented that gutka and betel quid were the causative agents most commonly observed among the study participants who were residents of Karachi.¹² The OSCCs were found to be more common in individuals with blue collar jobs, having low level of education, and who were chewers. Gutka-chewing was observed to be causing high incidence of buccal cancer compared to

betel quid.¹³ Kyo et al. documented the carcinogenic role of areca nut's metabolite in oral cancer formation.¹⁴ There are several biomarkers to identify the disease pattern and involvement. The biomarkers are present both at the level of tissue as well as body fluids. Biomarkers could be of different varieties, including proteomic, metabolomic and genomic. The biomarkers can be of valuable aid in diagnosis, metastasis and recurrence of cancer.¹⁵ There are various studies that documented the association of various genes, including TP53 (tumour protein p53), NOTCH1, CDKN2A (cyclin-dependent kinase inhibitor 2A), EGFR (epidermal growth factor receptor), and CCND1 (cyclin D1 protein) with HNSCC.¹⁶ NOTCH1 gene has its association with OSCC and is known to be associated with both oncogenic and tumour suppressor roles.¹⁷ A Chinese study also reported involvement of NOTCH1 pathway at various stages of OSCC development. It mentioned poor prognosis and life expectancy for such patients.¹⁵ Hijioka H. et al. using reverse transcriptase polymerase chain reaction (RT-PCR) showed that there was upregulation of NOTCH1 both in the tissue specimen as well as cell lines, suggesting that for OSCC treatment, the pathway of NOTCH1 should be inhibited.¹⁸

World over, there is variability in the development of OSCC along gender lines. Behera et al. found that OSCC was more common in males compared to females.¹⁹ A study in South Korea documented that OSCC was found in 67.2% males and 32.8% females.⁶ A study in Karachi, the largest city of Pakistan, documented the incidence to be 19.2%, with 67.8% males and 32.1% females.⁹

Keeping in mind the high morbidity and mortality of OSCC and the fact that cancer in late stages is difficult to counter, there is a clear need for early detection modalities. The OSCC then can be treated with appropriate therapies. The current narrative review was planned to determine the association of OSCC with NOTCH1 gene.

Methods, Results and Discussion

The narrative review was based on literature search for relevant articles published on Google Scholar, PubMed and Google. The inclusion criteria was original research articles published from 2013 to 2023 in English. Only those studies that documented detailed methodology and results were included. Key words used for the search were 'mutation', 'NOTCH1', 'gene', 'development', and 'Oral squamous cell carcinoma'. Articles published in any language other than English were excluded. Additionally, articles were excluded having ambiguous or incomplete results or methodology.

Of the 19 articles identified, 14(73.68%) were analysed in detail.

Procedures for finding NOTCH1 genetic mutations

There are various procedures by which NOTCH1's involvement in OSCC can be determined. Majority of the researchers used PCR for identifying this association, followed by immunohistochemistry (IHC), immunofluorescence staining, cell lines culture, Western blotting, wound-healing assay and invasion assay, while others used some uncommon tests, like Matrigel cell invasion assay, gamma-secretase inhibitors (GSI) treatment and transfection with short interfering ribonucleic acid (siRNA) (Table1). IHC supports the role of NOTCH1 gene in tumour progression and invasion. The cell lines of OSCC confirmed the presence of NOTCH1 signalling, and its absence (knockdown) resulted in inhibition of tumour cells proliferation. NOTCH1 mediates the tumour progression by activation of AKT (a serine/threonine-protein kinase) signaling.²⁰ Yoshida et al. revealed weak, modest and strong immunoreactions for NOTCH1 expression in normal, dysplastic and cancerous tissues, respectively. SAS cells (oral cancer cell line) treated with SiRNA showed low level of NOTCH1 gene expression compared to their controls, suggesting

Table: Association of NOTCH1 gene with development and of OSCC

Author	Year	Place	Number of participants/ cell lines/samples and Sampling procedures used	Methods for checking NOTCH1 association	Mutation/expression	Conclusion
Chung ⁵	2017	China	410 OSCC 282 Controls Blood samples	Genotyping using Sequenom MassARRAY System	Variant rs139994842 in exon 15 of NOTCH1 was significantly associated with an increased risk for OSCC	There was association between BQ chewing and NOTCH1 mutation leading to OSCC

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Song ⁷	2014	China	5113HNSCC Cell lines from Caucasian patients 51 tissue samples from Chinese patients of OSCC.	PCR	4 mutations out of 13 cell lines – 31% 152 nucleotide sequencing mutations in tumour tissue from Chinese patients. 120 (65%) synonymous variants 42(27%)- non synonymous somatic mutations 12 (8%)- insertion or deletion single nucleotide polymorphism	Notch 1 mutations were commonly found in OSCC in Chinese population and there is relation between these mutation sand clinical outcomes in OSCC patients
Yoshida ¹¹	2013	Japan	54 cancer tissue samples	PCR Cell lines Immunohistochemical staining Western blotting GSI Treatment Cell Proliferation Analysis Transfection with siRNA Immunofluorescence Microscopy Matrigel Cell Invasion Assay	NOTCH1 expressions were upregulated in patients with OSCC in the cell membrane of cells.	NOTCH1 is associated with cancer progression, metastasis. GSI is useful for inhibition of NOTCH1 pathway.
Kuo ¹⁴	2019	Taiwan	30 C57BL/6 mice divided into three groups. Each group had 10 mice	Immunohistochemistry Cell culture siRNA transfection methods Western blot analysis qRT-PCR	Cells treated with Arecoline N-oxide (areca nut) increase the somatic mutations of NOTCH1	There is high NOTCH1 activation when there is exposure to ANO leading to hyperplasia and cancer formation
Wu-Chau ¹⁵	2021	Taiwan	168 Tissue samples from OSCC patients	PCR Immunohistochemical staining	44 (26.19 %) NOTCH1 gene mutations	Progression of OSCC is associated with NOTCH1 expression
Uchibori ¹⁷	2017	Japan	40 Expression vectors HEK293 cell lines	Flow cytometry Wester blotting Immunofluorescence imaging Quantitative real-time PCR Cell growth assay Xenograft model	NOTCH1 mutation (p.A465T) in the ligand-binding region	NOTCH1 activity declined in A465T cells NOTCH1 gene having an oncogenic effect Mutation causes the loss of tumorigenicity by downregulating the NOTCH1 pathway.
Zheng ²⁰	2019	China	OSCC cell lines CAL27 and SCC9	Cell line culture Plasmid construction Transfection experiment Transwell invasion assays PCR Western blotting CCK-8 assays Immunofluorescence Flow cytometry Transwell invasion assays	Signaling pathway of EGFR–PI3K–AKT is activated by V1754L mutation	In OSCC, NOTCH1 is involved in EGFR–PI3K–AKT signaling pathway

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Elhendaway ²¹	2022	Egypt	44 Tumour specimen	Immunohistochemical staining	High expression of NOTCH1 in cancer tissue	High levels of Notch1 expression is related to poor prognostic outcomes
Ding ²²	2018	USA	78 Oral leukoplakia tissue samples 19 out of 78 from OSCC patients	Cell lines Immunohistochemical staining Western blotting Immunofluorescence	The rates of positive expression of Notch1 in normal mucosa, dysplasia and carcinoma were 20% (2/10), 64.7% (11/17) and 84.6% (11/13), respectively	NOTCH1 membranous expression is increased during OSCC
Salameti ²⁴	2019	UK	Tumour biopsy tissue-number not mentioned Normal mucosa of oral region – number not mentioned 15 SJG cell lines Tumour biopsy tissue Normal mucosa SJG cell lines	qRT-PCR Colony formation Cell migration Cell proliferation, differentiation and morphology Pharmacological inhibition of Notch signaling Gene expression microarrays Immunofluorescence microscopy Western blotting	Cell line SJG6-G-T mutation SJG6-C-T mutation SJG17-G-A mutation SJG41-C-T mutation	Inhibition of SERPINE1 via ETV7 facilitates tumour suppressive role of Notch1 gene
Ishida ²⁵	2013	Japan	3 cell lines HSC-2 HSC-4 Ca99-2	Quantitative real-time PCR Immunohistochemical staining Wound-healing assay Invasion assay	NOTCH1 mRNA expression was upregulated in HSC-2 and HSC-4 cells	NOTCH1 signaling induces epithelial to mesenchymal transition leading to OSCC
Zheng ²⁶	2018	China	1 CAL27 cell line Cell lines	Cell line culture PCR Western blotting Transfection with siRNA Immunofluorescence Flow cytometry Transwell invasion assays CCK-8 proliferation assays	NOTCH1 mutation leads to EGFR-PI3K/AKT signalling pathway	Abruptex domain mutations leads to proliferation and invasion in OSCC cell lines
Izumchenko ²⁷	2015	China	144 Tissue samples 50 OSCC samples 49 normal oral mucosa 45 leukoplakia biopsy samples	PCR	Mutations in 60% premalignant lesion and 54% OSCC	Premalignant lesions show NOTCH1 mutations. The mutations were commonly in Chinese population as compared to Caucasian cohort.
Aoyama ²⁸	2014	Japan	84 Tissue samples from OSCC patients Normal mucosa 6 OSCC cell lines	PCR	Cell line: in one cell line, G1243A (p.E415K), in EGF-like repeat 11 Tissue sample: 9.5% presented with point mutations Mutations in EGFr12, and EGFr10	Downregulation of NOTCH1 functions due to mutations in ligand.

the role of NOTCH1 in OSCC development. Notch1 gene signalling-related molecules were positive in all cell lines.¹¹

NOTCH1 receptors

NOTCH proteins are cell membrane receptors that are involved in cell growth, maturation, differentiation and apoptosis.¹⁹ NOTCH1 protein are heterodynamic proteins.²¹ There are three domains of NOTCH1 gene receptor; extracellular, transmembrane and intracytoplasmic. The extracellular domain is responsible for ligand binding, while the intracytoplasmic domain facilitates signal transduction. The extracellular domain is calcium-dependent. There are 5 NOTCH ligands: Delta Like Ligand-1 (DLL1), DLL3, DLL4, Jagged-1 (JAG1) and JAG2.^{11,22} Binding of ligand causes the extracellular cleavage at the juxtamembranous region. Cleavage of NOTCH intracellular domain (NICD) is facilitated by γ -secretase complex. The domains on intracellular region are RAM (RBP-J associated molecule), ANK (ankyrin) and C-terminal PEST (proline-, glutamic acid-, serine-, and threonine-rich) domain. The intracellular region goes to nucleus, RAM associates with CSL (CBF1, Suppressor of Hairless, Lag-1) and ANK associates with both CSL and transcriptional co-activator N-terminal end of mastermind-like 1 (MAML1) protein. This creates transcriptional complex.^{23,24} Uchibori et al. documented that ligand binding region of NOTCH1 receptor was mutated in OSCC.¹⁷ Most mutations occur at the epidermal growth factor (EGF)-like binding domain of NOTCH1.²⁴

Mechanism of NOTCH1 in tumourogenesis

The mechanism by which NOTCH1 is involved in development of OSCC is complex. Activation of NOTCH1 pathway induces epithelial-mesenchymal transition that results in transformation of epithelial cells to mesenchymal cells. The epithelial cells, when transformed, lose their cell polarity and cell junctional normal characteristics. Additionally, the cells become motile and spindle shaped. Epithelial-mesenchymal transition plays a pivotal role in cancer metastasis. The newly-developed cells then develop stem cell properties, inducing carcinogenesis. The epithelial characteristics of the cells become different as there is loss of epithelial markers, including E-cadherin. NOTCH1 signalling pathway has its connection with various processes, like fate of cell, their growth, maturation and apoptosis. The epithelial to mesenchymal transition plays a positive role in dissemination of tumour cells.²⁵ Yoshida et al.¹¹ documented the mechanism by which NOTCH1 gene is involved in OSCC, and revealed the presence of increased expression of NOTCH1 gene in the cell membrane of

affected stratified squamous epithelium of cancer region. The expression was lower in the normal tissue's basement membrane. The study also documented the role of tumour necrosis factor-alpha (TNF- α) and its linkage to NOTCH1. When there was high TNF- α , there was high NICD cleavage. Therefore, NOTCH1 was involved in increased tumour invasiveness as mediated by TNF- α . There is also a role of GSI in decreasing the tumour progression as it inhibits NOTCH1 pathway. This can help the researchers to go further so that drugs with GSI can be made available to combat the NOTCH1 pathway.¹¹

Zheng et al. also documented the role of NOTCH1 in facilitating the process of epithelial to mesenchyme transition. On the other hand, mesenchymal cells cause upregulation of mesenchymal markers, like vimentin and N-Cadherin.²⁶ SERPINE1 (serine proteinase inhibitor) is associated with increased metastasis. NICD is associated with downregulation of SERPINE1, leading to increased invasion.²⁴ A study in Egypt mentioned the role of JAG1 and NOTCH1 in tumourogenesis of OSCC. In tongue carcinoma, deregulation of NOTCH1/JAG1 facilitates cancer metastasis and advancement. Moreover, tumours that have both high expression of NOTCH1 and JAG1 expression have unfavourable survival rate.²¹

NOTCH1 mutations at the domain level and ethnicity

There is variability among different ethnicities around the world in terms of NOTCH1 mutations. The mutations are lower in the Western people (11-15%) compared to Chinese natives (54%).²⁷ A study conducted in Japan found the mutations to be present in 8 out of a sample size of 84.²⁸ A study on Caucasians revealed the involvement of NOTCH1 in somatic mutations, leading to OSCC. Studies indicated the involvement of extracellular domain containing EGF repeats. In the Asian population, Abruptex and ligand binding regions' mutations are responsible for OSCC, while in the Caucasians, ligand binding regions are involved in tumourogenesis. In Asian individuals, there was low prevalence of frameshift and missense mutation. There were more nonsense and indel mutations in Caucasians.²⁷ Song et al. compared mutations between Caucasians and Chinese. EGF repeat domain mutations were in 4 out of 6 nucleotide sequencing mutations. These also included a non-sense mutation at Abruptex region (AA 907-1143). Among the nucleotide sequencing mutations in Chinese samples, 1 was located at transcriptional activation domain (AA 2155-2374) and other at heterodimerisation domain (AA 1570-1734). About 40% substitutions were C>T, G>A, while A>G transitions were 24%. In total, there were G>T transversions. Also, 83% mutations were missense and

17% were non-sense.⁷ Aoyama et al. reported G>A transition mutations and there were substitution of amino acids.²⁸ A study in Taiwan documented mutations in various regions, including negative regulatory region (NRR), Ankyrin repeats region and EGF-like repeats region. Majority of the mutations were noticed in EGF-like repeats region, followed by NRR and Ankyrin repeats region. The ligand binding region mutations leads to downregulation of NOTCH1 function. The notch1 receptor lacking C receptor intracellular domain leads to nonsense and frameshift mutation, leading to transactivation of target genes. There were missense mutations in 53.7% individuals, followed by 18.5%, 16.7% and 1.9% frameshift, nonsense and inframe deletion, respectively.¹⁵ Aoyama et al. reported that all the mutations were nonsynonymous G>A transitions. The reported mutations were located at codon 378 (S>N), codon 376 (C>Y), codon 394 (G>D), codon 392 (V>I) and codon 387 (C>Y). At EGF-like repeat 10, there were 5 mutations, while EGF-like repeat 12 was involved in 1 mutation.²⁸ Song et al. documented that patients affected by NOTCH1 mutation leading to OSCC had a short period disease-free survival (DFS) rate²⁹. A study reported that most mutations are located in ligand binding site in the EGF-like repeats region. Even expression is high, and because of mutations, NICD are not formed and, therefore, frameshift and missense mutations result.¹⁵

The current narrative review highlighted the mechanism of NOTCH1 gene in the tumorigenesis of OSCC. There is an associated limitation. Since local research is lacking in the field of NOTCH1 gene's role in the development of cancer related to oral cavity, therefore, studies conducted elsewhere had to be depended upon for review. The current review is, therefore, a call to attention for Pakistani research community to reset its focus.

In Pakistan, OSCC is 4th among all cancers, and in Karachi it accounts for 19.2% of cancers.⁹ The high incidence of OSCC suggests that screening of the tumour should be done on a large scale and at multiple centres so that the findings may be generalised. As early diagnosis is associated with high survival rate, there is a need to make public aware of this deadly cancer. The tumour has both genetic and environmental linkage, so genetic mutations studies should be carried on patients of OSCC with tobacco smoking or chewing history and also on healthy adults with positive tobacco habits, but no cancer, so that one can find the association of NOTCH1 mutations and tobacco on the development of OSCC.

Conclusion

World over, NOTCH1 mutations are associated with the

development of OSCC. Most of the mutations are linked to ligand bind EGF-like repeat region of extracellular domain of NOTCH1. Once activated, the pathway is involved in tumour progression and metastasis. The Asians compared to the Caucasians are more affected by NOTCH1 mutations.

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Authors' Contributions

QJ: Conception and design, acquisition, analysis and interpretation of data, Drafting, critical revising, final

approval, accountability for all aspects of the work.

AU: Substantial contributions to the design of the work, acquisition, interpretation, final approval