

# A Computational Approach to Identify the Mutations in the Genes of the RTK Signaling Pathway and their Possible Association with Oral Squamous Cell Carcinoma

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## Abstract

**Background:** We aimed to determine the role of receptor tyrosine kinases (RTK) signaling family genes in the development of oral squamous cell carcinoma (OSCC).

**Method:** In the present in silico study, 40 whole genome sequences of patients with OSCC from the cBioPortal was analysed to identify the mutations in the genes of the RTK signaling pathway. Using the STRING v10.5, we further checked the gene with the highest frequency of mutations for its protein interactions. The obtained protein interaction network was used to identify the possible pathways related to disease phenotype.

**Results:** Epidermal growth factor receptor (EGFR) gene showed the highest frequency of mutation (5%) among the 16 genes clustered in the RTK signaling pathway as available in the cBioportal database. Missense mutations viz., G203E, R521K were identified in the EGFR gene. The other genes which returned positive results during analysis were *ERBB4* (D245N, L993S), *PDGFB* (R100H), and *PDGFRB* (L667M).

**Conclusion:** The in silico method of analysis can be a contemporary approach for identifying possible mutations or pathways associated with the development of OSCC. Further high throughput strategies should be applied to substantiate the role of the genes identified in the present study and draw conclusive evidence as to their association with the disease phenotype.

**Keywords:** Oral squamous cell carcinoma, Receptor tyrosine kinases, cBioportal, Mutations

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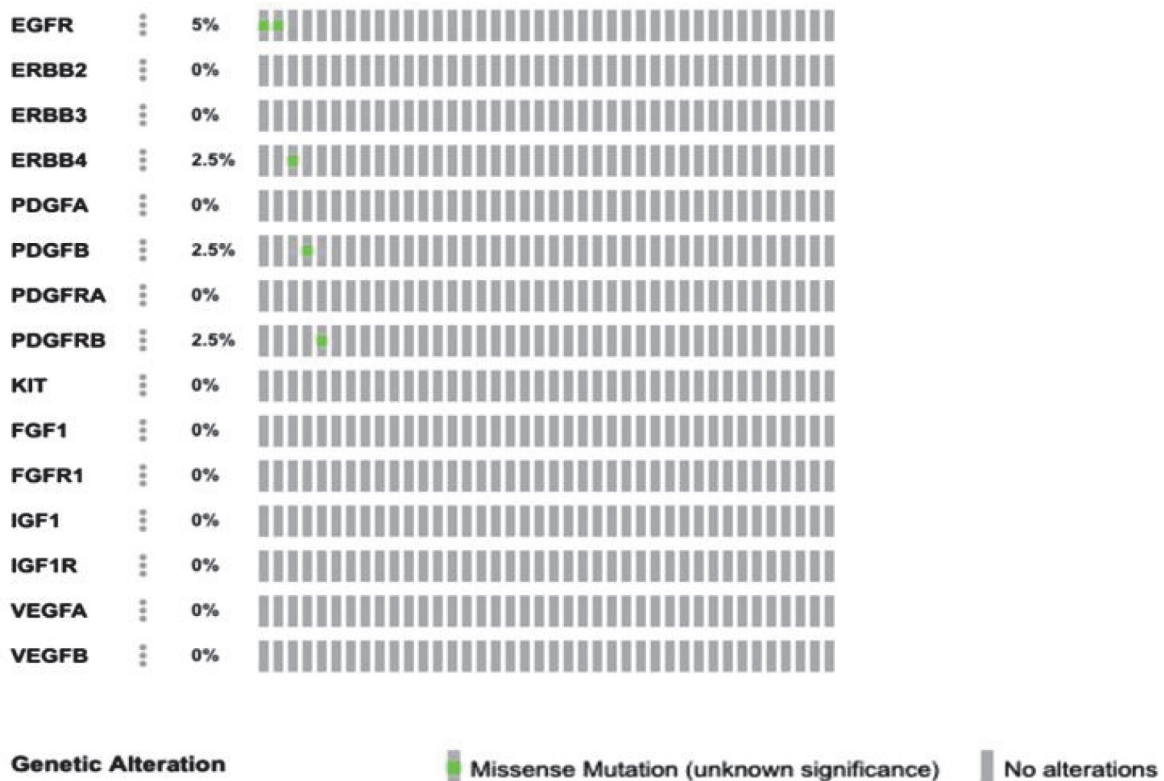
## Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant epithelial neoplasm affecting oral cavity and is capable of local destructive growth and distant metastasis.<sup>1, 2</sup> Carcinoma of squamous cell accounts for approximately 90% of all oral cancers.<sup>3</sup> It might influence any anatomical site in the mouth; however, it most commonly impacts the tongue and the floor of the mouth. This neoplasm usually arises from a pre-existing potentially malignant lesion. The use of tobacco and betel quid, heavy consumption of alcoholic beverages, and a diet low in fresh fruits and vegetables are well-known risk factors for OSCC. This disease is managed by surgery, chemotherapy, and radiation; however, regardless of the treatment modality, the five-year survival rate is poor at about 50%. This can be attributed to the fact that about two-thirds of people with OSCC already have a large lesion at the time of diagnosis.<sup>1</sup>

Worldwide, oral cancer accounts for

approximately 2%-4% of all cancer cases. In some regions, the prevalence of oral cancer is higher, reaching around 45% of all cancers in India.<sup>4</sup> Despite the advances in therapeutic approaches, the percentages associated with the morbidity and mortality of OSCC have not considerably improved over the past 30 years. The percentages of mortality and morbidity are 6.6/100,000 and 3.1/100,000 in males; while, 2.9/100,000 and 1.4/100,000 in females, respectively.<sup>5</sup> Additionally, the incidence of OSCC is growing among young white individuals aged 18-44 years, particularly among white women.<sup>6</sup> Regardless of the simple access of oral cavity for clinical examination, OSCC is often diagnosed in advanced stages. The most common reasons are the initial wrong diagnosis and the ignorance of the patient or the attending physician.<sup>7</sup>

The receptor tyrosine kinases (RTK) family comprises cell surface receptors for growth factors and signaling molecules. There exist several subfamilies, including epidermal growth factor receptors (EGFRs), fibroblast growth factor



**Figure 1.** Oncoprint data show alterations in the genes involved in receptor tyrosine kinases signaling pathway in the oral squamous cell carcinoma cases.

receptors (FGFRs), insulin and insulin-like growth factor receptors (IR and IGFR), platelet-derived growth factor receptors (PDGFRs), vascular endothelial growth factor receptors (VEGFRs), hepatocyte growth factor receptors (HGFRs), and proto-oncogene c-KIT.<sup>8,9</sup> Most RTKs are found mutated in a variety of cancers and from different tissue origins. RTKs are high-affinity cell surface receptors for many cytokines, hormones, and polypeptide growth factors. These are key regulators of critical cellular processes such as proliferation and differentiation, cell migration, cell survival and metabolism, and cell cycle control.<sup>10-12</sup> About 58 RTKs have been identified in the human genome, all of which fall under 20 families.<sup>13</sup> The EGFR (EGFR/ErbB1/Her1) is a member of ErbB/EGFR receptor family and is a crucial transforming RTK in the head and neck squamous cell carcinoma (HNSCC), including OSCC.<sup>14</sup> Incidence and progression of HNSCC are correlated with habitual usage of tobacco which entails the release of nicotine.<sup>15</sup> Nicotine triggers the secretion of epidermal growth factor. It binds to EGFR and activates the PI3K-AKT, RAS-MEK-ERK, and JAK-STAT signaling pathways; therefore, it promotes the proliferation and survival of cancer cells.<sup>16</sup> In addition, activation of genetic aberrations such as amplification and mutation of EGFR are found in approximately 15% of human papillomavirus-negative [HPV (-)] HNSCC such as OSCC.<sup>14</sup> The present study investigated the genetic alterations in the RTK family genes via an in silico approach. This study is the first of its kind reporting the frequency and type of mutations in the genes of RTK signaling pathway and providing a clue on the putative association of these genes with OSCC.

**Table 1.** The demographic details of cases in the oral squamous cell carcinoma (MD Anderson, Cancer Discov 2013) dataset

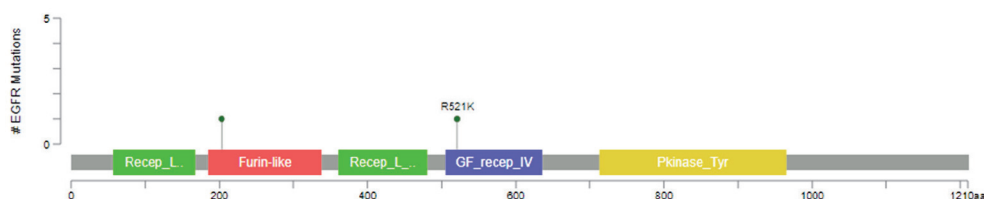
| Demographic Features                         | Cases N=40 (%)  |
|--|---|
| Gender distribution<br>(Male : Female ratio) | Male = 28 (70%)<br>Female = 12 (30%)<br>(2.3:1)       |
| Diagnosis age                                | 26-85 years   |
| HPV status                                   | Positive : 1<br>Negative : 11<br>Not detected : 28    |
| Smoking status                               | Smoker : 29 (72.5%)<br>Non - smoker : 11 (27.5%)      |
| Daily alcohol                                | Alcoholic : 9 (22.5%)<br>Non - alcoholic : 31 (77.5%) |
| Mutation count                               | 10-173  |

## Subjects and Methods

In the present study, we analysed 40 whole genome sequences from OSCC patients to identify the mutations in the genes of the RTK pathway. The genome sequence data was retrieved from cBioportal. The frequency of mutations and their putative associations OSCC were ascertained using text mining process.

### Sample data set

The cBioPortal for Cancer Genomics (<http://cbioportal.org>) integrates an exhaustive collection of molecular profiling information from cancer tissues and cell lines.<sup>17,18</sup> The database is user-friendly and hosts genetic, epigenetic, and proteomic information of the cases registered. The sample data set includes the sequence information of 40 OSCC cases, which is used in the present study. Demographic details of the cases in the OSCC (MD Anderson, Cancer Discov 2013) dataset were recorded.<sup>19</sup>



**Figure 2.** This figure shows mutations located in the epidermal growth factor receptor gene.

### Mutation Analysis

We initiated a single query for mutation analysis by selecting the OSCC cases from the cBioPortal database. The case set included 40 sequenced tumors analysed for mutations in genes associated with RTK signaling pathway. The gene cluster includes *EGFR* (Epidermal growth factor receptor), *ERBB2*, *ERBB3*, *ERBB4* (Erb-B2 receptor tyrosine kinase 2, 3, 4), *PDGFA* (Platelet derived growth factor subunit A), *PDGFB* (Platelet derived growth factor subunit B), *PDGFRA* (Platelet derived growth factor receptor subunit A), *PDGFRB* (Platelet derived growth factor receptor subunit B), *KIT* (Tyrosine-protein kinase Kit), *FGF1* (Fibroblast growth factor 1), *FGFR1* (fibroblast growth factor receptor 1), *IGF1* (Insulin-like growth factor 1), *IGF1R* (Insulin-like growth factor receptor 1), *VEGFA* (Vascular endothelial growth factor A), *VEGFB* (Vascular endothelial growth factor B), and *KDR* (Kinase insert domain receptor). The gene cluster was predefined and selected from the menu available in the database. Moreover, we observed that these genes were associated with many cancer types through exhaustive literature survey.

### OncoPrint data

Submission of query returned a window with OncoPrint data, indicating the presence of mutations in crucial genes associated with RTK signaling pathway. We further documented the somatic mutation frequency and the mutation site in the candidate genes.

### Protein network interactions

We assessed the interactions of the protein encoded by the gene with the highest mutation frequency via submitting the query protein in the STRING v10.5 pipeline.<sup>20</sup>

### Text mining

We employed the information from OncoPrint as a preliminary data to acquire further reports on the association of gene mutations identified with OSCC cases. A text mining approach was followed to consolidate the reports from various populations worldwide.

### ExAC data analysis

The exome aggregation consortium (ExAC) provides sequence data of 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies. We extracted these sequences for public release based on consent, consortium permission, exome data quality, and lack of relatedness with other samples. The ExAC genome data was employed to compare the observed mutations documented in the present study with that of the reported mutations deposited in the ExAC repository.<sup>21</sup>

### Protein stability analysis

I-Mutant v3.0 is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single point mutations. The software's predictions were based on the protein sequence. We classified the predictions into three classes: neutral mutation ( $-0.5 \leq \text{DDG} \leq 0.5$  kcal/mol), large decrease ( $< -0.5$  kcal/mol), and large increase ( $> 0.5$  kcal/mol). The free energy change (DDG) predicted by I-Mutant 3.0 is based on the difference between unfolding Gibbs free energy change of mutant and native protein (kcal/mol).<sup>22</sup>

### MutPred analysis

MutPred v2 is a standalone and web application developed to categorize amino acid substitutions as pathogenic or benign in human. We used the wild-type protein sequence in FASTA format (<https://www.ncbi.nlm.nih.gov/protein/>) and substituted the variants at the sites defined in oncoPrint data. The probability of the mutation being deleterious was then reported (<http://mutpred.mutdb.org/>).<sup>23</sup>

## Results

The OSCC dataset obtained from the cBioPortal site included 40 completely sequenced samples from patients with a diagnosis age of 26-85 years. The demographic details were made available for the users in the cBioPortal database. The number of male participants (70%) was more in the study group compared to females (30%). Among the 40 individuals, 72.5% were smokers,

**Table 2.** The list of genes carrying mutations involved in RTK signaling pathway in oral squamous cell carcinoma patients [US: unknown significance]

| Gene  | Type of mutation | Frequency | Amino acid change | ExAC analysis       | Mutant protein stability analysis | Pathogenicity analysis |
|---|------------------|-----------|-------------------|---------------------|-----------------------------------|------------------------|
| <i>EGFR</i><br>Epidermal growth factor receptor isoform A precursor | Missense (US)    | 5%        | G203E<br>R521K    | Novel<br>rs2227983* | Decrease<br>Decrease              | 0.726**<br>0.073       |
| <i>ERBB4</i><br>ErbB2 receptor tyrosine kinase 4                    | Missense (US)    | 2.5%      | D245N<br>L993S    | Novel<br>Novel      | Decrease<br>Decrease              | 0.515**<br>0.848**     |
| <i>PDGFB</i><br>Platelet derived growth factor                      | Missense (US)    | 2.5%      | R100H             | Novel               | Decrease                          | 0.636**                |
| <i>PDGFRB</i><br>Platelet derived growth factor receptor            | Missense (US)    | 2.5%      | L667M             | Novel               | Decrease                          | 0.598**                |

\*SNP: single nucleotide polymorphism identified using ExAC.; \*\*Pathogenicity of mutations identified using MutPred; RTK: Receptor tyrosine kinases

27.5% were non-smokers, and 22.5% were alcoholic. The human papilloma virus (HPV) statuses of the twelve participants were recorded, out of which one was positive and the others tested negative (Table 1). The query submitted in the cBioPortal pipeline produced results revealing mutations in the *EGFR*, *ERBB4*, *PDGFB*, *PDGFRB* genes. All the gene alterations were missense mutations, where one amino acid is substituted by the other. Mutation frequency was observed to be highest in the *EGFR* gene (5%), while other genes showed the same mutation frequency (2.5%). Missense mutation in the *EGFR* gene converts glycine to glutamic acid (G203E) and arginine to lysine (R521K). Also, missense mutation in *ERBB4* gene converts aspartic acid to asparagine (D245N) and leucine to serine (L993S). Arginine to histidine (R100H) and leucine to methionine (L667M) were the mutations observed in *PDGFB* and *PDGFRB* genes,

respectively (Figures 1 and 2).

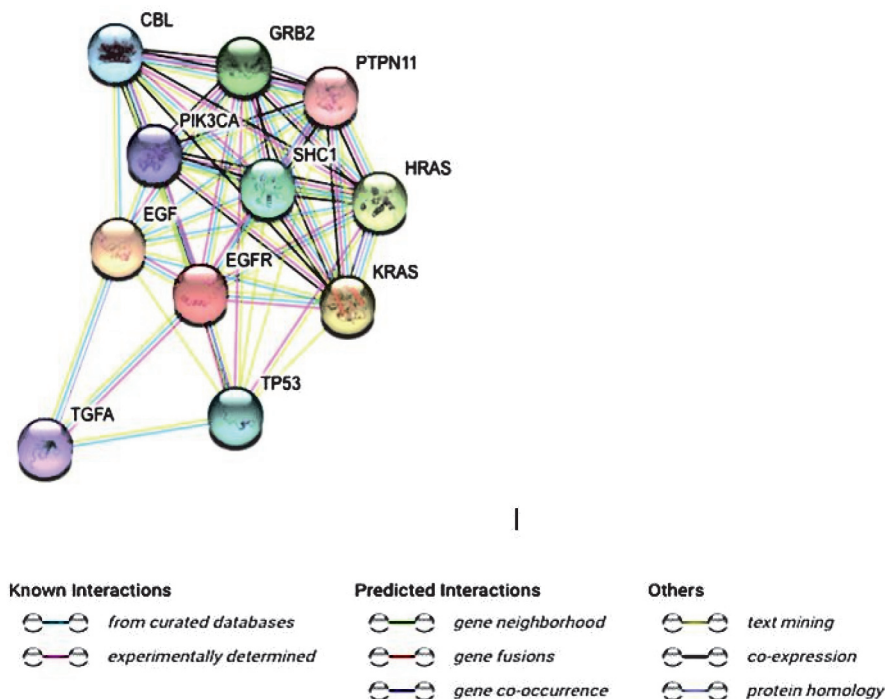
The protein interaction network reveals the major interactions of *EGFR* with genes such as *TP53*, *KRAS*, *HRAS*, *PTPN11*, *EGF*, *TGFA*, *PIK3CA*, *CBL*, *GRB2*, and *SHC1*. These are crucial regulators of tumour suppression and signal transduction pathways. Protein encoded by TP53, a tumour suppressor, is activated by diverse cellular stresses and regulates the expression of target genes. Therefore, it induces cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Several other proto-oncogenes such as Kirsten ras (*KRAS*) and Harvey ras (*HRAS*) encode proteins with essential roles in signal transduction pathways. Cas-BR-M ligase (*CBL*) gene is also a proto-oncogene acting as a negative regulator of many signal transduction pathways. The protein encoded by *PTPN11* gene, a member of the protein tyrosine phosphatase (PTP) family, is known to be a signaling molecule

which regulates a variety of cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation. The *EGF* protein functions as a potent mitogenic factor and *TGFA* and *PIK3CA* genes encode growth factor that plays an important role in the growth, proliferation, and differentiation of numerous cell types. All of these cell types were found to activate the signaling pathway for cell proliferation, differentiation, and development. Moreover, *PIK3CA* has been implicated as an oncogene. *GRB2* and *SHC1* are factors associated with signal transduction pathway. The knowledge about the genes in the interaction cascade and the functional mutations observed in the key identified genes will provide a clue as to the underlying molecular mechanisms of OSCC (Figure 3). Protein stability analysis, performed via I-Mutant software, revealed that the substitution of amino acid decreased the stability of the protein in all the mutation encoded proteins. Additionally, the MutPred score identified several mutations to be highly pathogenic (score >0.50) (Table 2).

## Discussion

Genetic variations such as single nucleotide variants (SNVs) and copy number variants have long been associated with oral cancer and other devastating diseases. The present study identified *EGFR* mutations in about 5% of patients with OSCC. We observed a novel variant and a reported variant (*rs2227983*) to influence the protein stability and pathogenicity of the mutant protein. Other genes in the RTK pathway also showed similar changes which reduced the stability and increased the pathogenicity of the mutant proteins. Analysis of genes involved in crucial pathways demonstrated nearly 70.2% alteration in the RTK/MAPK/PI3K pathway.<sup>24</sup> *EGFR* polysomy and amplification was reported to be significantly associated with lymph node metastasis.<sup>25</sup> A cohort and systematic review analysis on the prevalence of *EGFR* mutation revealed 159 *EGFR* tyrosine kinase domain mutations in approximately 4122 patients with HNSCC, accounting for about 2.8% of the overall prevalence.<sup>26</sup>

Overexpression of *EGFR* exists in up to 90% of HNSCC and is thought to be involved in carcinogenesis and metastasis.<sup>27</sup> The degree of



**Figure 3.** This figure shows protein interaction network of epidermal growth factor receptor gene.

EGFR expression correlates with phenotype aggression, increased resistance to treatment, and poor clinical outcomes.<sup>28, 29</sup> Although several studies have reported the overexpression of EGFR and Her-2 in head and neck cancers, the clinical relevance of the finding was found to vary. Storkel et al. found that the over expression of EGFR was associated with shortened survival.<sup>30</sup> On the other hand, Werkmeister et al. reported that Her-2 was strongly related to survival.<sup>31</sup> Christensen et al. and Khan et al. were not able to find any significant correlation between either EGFR of Her-2 and clinicopathological features or prognosis.<sup>32, 30</sup> However, available reports showed the co-localisation of both molecules in oral cancer tissues. Further, the combined use of these biomarkers was also regarded as a strong predictor for cancer prognosis.<sup>33, 34</sup> A recent study by Mirza et al. provided substantial evidence on the association between high expression levels of EGFR and OSCC in a Pakistani population.<sup>35</sup> The study findings showed that 51% of patients with oral premalignant lesions and 67% of OSCC patients had increased levels of EGFR expression. In addition, the upper and lower lip lesions had the highest frequency of EGFR positivity, low survival rates, and high chances of recurrence.<sup>36</sup> In a similar study by Oikawa et al., nearly 16.8% of OSCC patients showed amplified gene encoding RTK.<sup>37</sup> Furthermore, distance metastasis was identified in 24% of patients with an abnormality in RTK genes. The survival rate was found to be influenced by the presence of gene mutations in RTK pathway (64.6%) as compared to no-RTK amplification group, thus, the providing conclusive evidence regarding the vital role of RTK signaling genes in the pathogenesis and progression of OSCC.

Similar to *EGFR* and *PDGF*, *PDGFRs* play important roles in the regulation of cell growth and survival. Mutations within *PDGFR $\alpha$*  gene have been reported in 5% of gastrointestinal stromal cancer. These mutations affected tyrosine kinase domains and juxta-membrane domain.<sup>38</sup> *PDGFR* genes were further involved in gene rearrangements found in certain leukemias.<sup>39</sup> In addition, *PDGFR $\alpha$*  amplifications were observed

in 5%-10% of glioblastoma multiforme in oligodendrocytoma, esophageal squamous cell carcinoma, and artery intimal sarcomas.<sup>40-42</sup> As for other dysfunctional RTKs, tyrosine kinase inhibitors such as imatinib, sunitinib, sorafenib, pazopanib, and nilotinib have been developed to target PDGFR directly or as a secondary target. Briefly, the genetic variations identified in the present study revealed the pathogenicity of mutations and warrants validation of these variants in the Indian population by use of genotyping methods.

## Conclusion

In silico analysis employed in the present study was successful in identifying novel and reported mutations in genes possibly associated with a disease phenotype. The present study unraveled the possible relationship of mutations in RTK signaling genes with OSCC. Further probing into this pathway may open new avenues into the identification of candidate genes which may be targeted to design drugs specific for OSCC phenotype. As a major limitation related to population bias, the samples in the present study did not represent all the ethnic groups or populations from around the world. Accordingly, it is imperative that future studies analyze the documented variations in these putative genes across several ethnic groups to substantiate the association of the genes with OSCC.

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## Conflicts of Interest

None declared.

## References

1. Markopoulos AK. Current aspects on oral squamous cell carcinoma. *Open Dent J.* 2012;6:126-30. doi: 10.2174/1874210601206010126.
2. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. *Oncol Rev.* 2014;8(1):244. doi: 10.4081/oncol.2014.244.
3. George A M, George R. Incidence of level IV nodal metastasis in oral squamous cell carcinoma- A retrospective study in South Indian patients presenting in an oral cancer institute. *J Adv Pharm Edu Res.* 2017;7(3):300-2.
4. Siddiqui IA, Farooq MU, Siddiqui RA, Rafi S MT. Role of toluidine blue in early detection of oral Cancer. *Pak J Med Sci.* 2006;22:184-7. doi:10.1097/MOO.0b013e32832771da
5. Mehrotra R, Yadav S. Oral squamous cell carcinoma: etiology, pathogenesis and prognostic value of genomic alterations. *Indian J Cancer.* 2006;43(2):60-6. doi: 10.4103/0019-509X.25886
6. Patel SC, Carpenter WR, Tyree S, Couch ME, Weissler M, Hackman T, et al. Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. *J Clin Oncol.* 2011; 29(11): 1488-94. doi: 10.1200/JCO.2010.31.7883.
7. Scott S E, Grunfeld E A, Main J, Mc Gurk M. Patient delay in oral cancer: a qualitative study of patients' experiences. *Psychooncology.* 2006;15(6):474-85.
8. Li E, Hristova K. Role of receptor tyrosine kinase transmembrane domains in cell signaling and human pathologies. *Biochemistry.* 2006;45(20):6241-51. doi:10.1021/bi060609y.
9. Hubbard S R, Miller W T. Receptor tyrosine kinases: Mechanisms of activation and signaling. *Curr Opin Cell Biol.* 2007; 19: 117-23. doi: 10.1016/j.ceb.2007.02.010.
10. Blume-Jensen P, Hunter T. Oncogenic kinase signaling. *Nature.* 2001;411(6835):355-65. doi: 10.1038/35077225.
11. Grassot J, Mouchiroud G, Perrière G. RTKdb: database of Receptor Tyrosine Kinase. *Nucleic Acids Res.* 2003; 31(1): 353-8. doi: 10.1093/nar/gkg036.
12. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 1990;61(2):203-12. doi: 10.1016/0092-8674(90)90801-k.
13. Lemmon M A, Schlessinger J. Cell signalling by receptor tyrosine kinases. *Cell.* 2010; 141(7): 1117-34. doi: 10.1016/j.cell.2010.06.011.
14. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature.* 2015; 517(7536): 576-82. doi: 10.1038/nature14129.
15. Schaal C, Chellappan S P. Nicotine-mediated cell proliferation and tumor progression in smoking-related cancers. *Mol Canc Res.* 2014;12: 14-23. doi: 10.1158/1541-7786.MCR-13-0541.
16. Grando SA. Connections of nicotine to cancer. *Nat Rev Cancer.* 2014;14(6):419-29. doi: 10.1038/nrc3725.
17. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2012;2(5):401-4. doi: 10.1158/2159-8290.CD-12-0095.
18. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* 2013; 6(269):p11. doi: 10.1126/scisignal.2004088.
19. Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov.* 2013;3(7):770-81. doi: 10.1158/2159-8290.CD-12-0537.
20. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* 2017;45(D1):D362-D368. doi: 10.1093/nar/gkw937.
21. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016; 536: 285-91. doi: 10.1038/nature19057.
22. Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Res.* 2005;33(Web Server issue):W306-10. doi: 10.1093/nar/gki375.
23. Pejaver V, Urresti J, Lugo-Martinez J, Pagel KA, Lin GN, Nam H, et al. MutPred2: inferring the molecular and phenotypic impact of amino acid variants. *bioRxiv.* 134981.
24. Nakagaki T, Tamura M, Kobashi K, Koyama R, Fukushima H, Ohashi T, et al. Profiling cancer-related gene mutations in oral squamous cell carcinoma from Japanese patients by targeted amplicon sequencing. *Oncotarget.* 2017;8(35):59113-22. doi: 10.18632/oncotarget.19262.
25. Huang SF, Chien HT, Cheng SD, Chuang WY, Liao CT, Wang HM. EGFR copy number alterations in primary tumors, metastatic lymph nodes, and recurrent and multiple primary tumors in oral cavity squamous cell carcinoma. *BMC Cancer.* 2017;17(1):592. doi: 10.1186/s12885-017-3586-9.
26. Perisanidis C. Prevalence of EGFR tyrosine kinase domain mutations in head and neck squamous cell carcinoma: Cohort study and systematic review. *In Vivo.* 2017;31(1):23-34. doi: 10.21873/invivo.11020.



27. Temam S, Kawaguchi H, El-Naggar AK, Jelinek J, Tang H, Liu DD, et al. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J Clin Oncol.* 2007;25(16):2164-70. doi: 10.1200/JCO.2006.06.6605.
28. Pomerantz R G, Grandis J R. The epidermal growth receptor signalling network in head and neck carcinogenesis and implications for targeted therapy. *Semin Oncol.* 2004;31: 734-43. doi: 10.1053/j.seminoncol.2004.09.015.
29. Pernas FG, Allen CT, Winters ME, Yan B, Friedman J, Dabir B, et al. Proteomic signatures of epidermal growth factor receptor and survival signal pathways correspond to gefitinib sensitivity in head and neck cancer. *Clin Cancer Res.* 2009;15(7): 2361-72. doi: 10.1158/1078-0432.CCR-08-1011.
30. Storkel S, Reichert T, Reiffen K A, Wagner W. EGFR and PCNA expression in oral squamous cell carcinomas – a valuable tool in estimating the patient's prognosis. *Eur J Cancer B Oral Oncol.* 1993;29B(4):273-7.
31. Werkmeister R, Brandt B, Joos U. Clinical relevance of erbB-1 and -2 oncogenes in oral carcinomas. *Oral Oncol.* 2000;36(1):100-5.
32. Christensen ME, Engbaek F, Therkildsen MH, Bretlau P, Nexø E. A sensitive enzyme-linked immunosorbent assay used for quantitation of epidermal growth factor receptor protein in head and neck carcinomas: evaluation, interpretations and limitations. *Br J Cancer.* 1995; 72: 1487-93.
33. Khan AJ, King BL, Smith BD, Smith GL, DiGiovanna MP, Carter D, et al. Characterization of the HER-2/neu oncogene by immunohistochemical and fluorescence in situ hybridization analysis in oral and oropharyngeal squamous cell carcinoma. *Clin Cancer Res.* 2002;8(2):540-8.
34. Bei R, Pompa G, Vitolo D, Moriconi E, Ciocci L, Quaranta M, et al. Co-localization of multiple ErbB receptors in stratified epithelium of oral squamous cell carcinoma. *J Pathol.* 2001;195(3):343-8. doi: 10.1002/path.965.
35. Xia W, Lau YK, Zhang HZ, Xiao FY, Johnston DA, Liu AR, et al. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. *Clin Cancer Res.* 1999;5(12):4164-74.
36. Mirza Y, Ali SMA, Awan MS, Idress R, Naeem S, Zahid N, et al. Overexpression of EGFR in oral premalignant lesions and OSCC and its impact on survival and recurrence. *Oncomedicine.* 2018;3:28-36. doi:10.7150/oncm.22614.
37. Oikawa Y, Morita KI, Kayamori K, Tanimoto K, Sakamoto K, Katoh H, et al. Receptor tyrosine kinase amplification is predictive of distant metastasis in patients with oral squamous cell carcinoma. *Cancer Sci.* 2017;108(2):256-66. doi: 10.1111/cas.13126.
38. Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, et al. PDGFRA mutations in gastrointestinal stromal tumors: Frequency, spectrum and in vitro sensitivity to imatinib. *J Clin Oncol.* 2005; 23(23):5357-64. doi: 10.1200/JCO.2005.14.068.
39. Toffalini F, Demoulin JB. New insights into the mechanisms of hematopoietic cell transformation by activated receptor tyrosine kinases. *Blood.* 2010; 116(14):2429-37. doi: 10.1182/blood-2010-04-279752.
40. Fleming TP, Saxena A, Clark WC, Robertson JT, Oldfield EH, Aaronson SA, et al. Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. *Cancer Res.* 1992;52(16):4550-3.
41. Kumabe T, Sohma Y, Kayama T, Yoshimoto T, Yamamoto T. Amplification of alpha-platelet-derived growth factor receptor gene lacking an exon coding for a portion of the extracellular region in a primary brain tumor of glial origin. *Oncogene.* 1992;7(4):627-33.
42. Puputti M, Tynninen O, Sihto H, Blom T, Mäenpää H, Isola J, et al. Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. *Mol Cancer Res.* 2006;4(12):927-34. doi: 10.1158/1541-7786.MCR-06-0085.