RESEARCH ARTICLE

Lack of Mutations in Protein Tyrosine Kinase Domain Coding Exons 19 and 21 of the EGFR Gene in Oral Squamous Cell Carcinomas

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Abstract

Background: The epidermal growth factor receptor (EGFR) plays a vital role in the activation and inactivation of receptor tyrosine kinases. Mutations in exons 19 and 21 of EGFR are commonly found to be associated with non small cell lung carcinoma and triple negative breast cancer, enhancing sensitivity to EGFR targeting chemotherapeutic agents. Since amplification and prolonged activation of EGFR molecules have been identified in oral squamous cell carcinomas (OSCC), we investigated whether OSCCs carried mutations in exons 19 and 21 of EGFR to their incidence. Materials and Methods: Tumor chromosomal DNA isolated from forty surgically excised oral squamous cell carcinoma tissues was subjected to PCR amplification with intronic primers flanking exons 19 and 21 of the EGFR gene. The PCR amplicons were subsequently subjected to direct sequencing to elucidate the mutation status. Results: Data analysis of the EGFR exon 19 and 21 coding sequences did not show any mutations in the forty OSCC samples that were analyzed. Conclusions: To the best of our knowledge, this is the first study to have investigated the genetic status of exons 19 and 21 of EGFR in Indian OSCCs and identified that mutation in EGFR exon 19 and 21 may not contribute towards their genesis. The absence of mutations also indicates that oral cancerous lesions may not be as sensitive as other cancers to chemotherapeutic agents targeting EGFR.

Keywords: EGFR mutation - oral carcinoma - exon 19 - exon 21 - India

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Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that belongs to the Erb family of proteins, known to transduce promitotic signals emanating from the membrane to the nucleus. The mature form of EGFR (without signal peptide) has 28 exons that encodes a 1210 amino acid long protein containing an extracellular ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. Upon binding of the pro-mitotic ligand, epidermal growth factor (EGF), to its extracellular domain, EGFR molecules form homo- and hetero-dimers with its fellow and other members of Erb family. Dimerization in turn triggers autophosphorylation of specific residues such as Tyrosine 845, Tyrosine 1068, Tyrosine 1086, Tyrosine 1072 and others within the cytoplasmic domains of the associated molecules that enables them to assume active conformation (Huang and Chang, 2011). Activated EGFR subsequently propagates the pro-mitotic signal to the nucleus via a cascade of activation events that include activation of the Ras (a G-protein), Raf, Mek and Erk1/2 (Tushar and Ramanathan, 2013; Valiathan et al., 2012). Following transduction of the requisite signals, the activated EGFR molecules are degraded by processes called clathrin mediated endocytosis (CME) and/or non-clathrin mediated endocytosis (NCE) (Woelk et al., 2007; Tushar and Ramanathan, 2013). Termination of EGFR activation results in effective cessation of promitotic signals. The NCE pathway is mediated by an ubiquitin ligase called c-Cbl, which binds to phosphorylated 1045 tyrosine residue of EGFR coded by exon 27 (Pennock and Wang, 2008). This region was previously studied in thirty-five oral squamous cell carcinoma samples of which none showed any mutation in the region (Tushar and Ramanathan, 2013).

EGFR apart from being involved in cell apoptosis, proliferation, division and differentiation, also acts as a transcriptional co-activator for seven oncogenic genes namely, cyclin D1, cyclooxygenase, Aurora kinase A, B-Myb, nitric oxide synthase, breast cancer resistant protein and c-Myc (Brand et al., 2011). Hence, an aberrant EGFR activity may be expected to cause functional dysregulation of the oncogenic genes that are transcriptional targets of nuclear EGFR. Indeed, overexpression of a few of the above mentioned oncogenes

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has been shown to be associated with mutant EGFR allele. For example, overexpression of the cell cycle promoter cyclin D1 gene has been observed in lung cancer tissues and cell lines carrying mutant EGFR allele (Sasaki et al., 2007). Similarly, overexpression of the cytochrome c oxidase, subunit 2, (COX2) has been reported in glioblastomas carrying mutant EGFR allele (Lo et al., 2010). While such association studies do not exist in oral squamous cell carcinoma (OSCC) lesions, an independent investigation has identified overexpression of COX2 to be related with the clinical stage of the OSCC lesions (Li and Cui, 2013).

EGFR mutations have become increasingly prominent in cancers including but not limiting to head and neck cancer, brain cancer, breast cancer, oral cancer, lung cancer and others. Extensive research has confirmed exon 19 and exon 21, that code for part of tyrosine kinase domain of EGFR in several cancers, to be the hot spot for activating mutations. Almost 90% of EGFR mutations are either missense mutations in exon 21 or deletions in exon 19 (D'Angelo et al., 2010). Consistently, an overall incidence of 48-83% and 43-86% of NSCLC tissue samples has been reported to carry exon 19 deletions and L858R missense mutation in exon 21 respectively (Asahina et al., 2006; Inoue et al., 2006; Mitsudomi and Yatabe., 2010). Though other mutation events have been reported both in exon 19 and 21, they however, occur at a lesser frequency which includes insertion in exon 19 in 1% and L861Q missense mutation in exon 21 in 2% of NSCLCs. More recently these mutations were also reported in 5.7% and 2.8% of triple negative breast cancer, respectively (Teng et al., 2011). These data led us to investigate whether mutations in exon 19 and 21 of EGFR gene occurred in oral squamous cell carcinoma (OSCC) tissues.

Materials and Methods

Study design and subjects

The occurrence of mutations in the exon 19 and 21 of EGFR was investigated in a cross sectional study on well differentiated oral squamous cell carcinoma tissue samples that were obtained post-surgically from patients visiting tertiary cancer hospitals in Tamil Nadu. Of the forty samples that were analyzed, thirty five of them were investigated earlier (Tushar and Ramanathan, 2013), while five of them were new samples. The study was approved by the institutional ethics committee (IEC) and was conducted at the Human Genetics Laboratory of Sree Balaji Medical and Dental College and Hospitals.

Tumor gDNA extraction, PCR amplification and direct sequencing

Surgically excised biopsies of oral squamous cell carcinoma were collected and transported in RNA Later (Cat#76106, Qiagen, USA). Genomic DNA extraction was processed as described earlier (Jayaraman et al., 2012, Valiathan et al., 2012). Intronic primers were used to amplify exon 19, 19F2 (Forward primer): gccagttaacgtcttccttc and 19R2 (Reverse primer): ccagacatgagaaaaggtggg, and exon 21, 21F (Forward primer): gcctttccattctttggatcag and 21R (Reverse primer): caatacagctagtgggaaggc, of the EGFR gene, using 25ng of genomic DNA. The exon 19 and 21 were amplified under the following conditions: after an initial denaturation at 94°C for 2 min, the samples were subjected to 35 cycles of denaturing at 95°C for 30 sec, annealing at 55°C for 45 sec, extension at 72°C for 45 sec, which was then followed by a final extension at 72°C for 5 min as described earlier (Rajendran et al., 2013). The PCR amplicons were run in a 1.5% agarose gel and subjected to SAP treatment before being sequenced.

Results

The protein kinase domain of EGFR plays a vital role in the regulation of cellular apoptosis, differentiation, division and proliferation. The exons 18 to 21 of EGFR are involved in encoding this domain of the EGFR. Mutations in any of these exons may be expected to affect the protein kinase function of EGFR. Indeed, a large number of NSCLC and triple negative breast cancers have been found to carry deletions of exon 19 and missense mutations in exon 21. The genomic DNA extracted from 40 oral squamous cell carcinoma tissue samples were amplified using intronic primers flanking exon 19 and 21 of EGFR. The amplified products were then subjected to direct sequencing analysis, which showed no mutations in exon 19 and 21.

Discussion

Functional deregulation of EGFR due to mutations in coding exons and copy number amplification is the most common event in cancers, especially among receptor tyrosine kinases. While copy number amplification and activating truncated mutations (EGFRvlll) have been observed in OSCCs, exon mutations as those that occur in NSCLCs or triple negative breast cancers have not yet been reported. In order to address this issue, we investigated the genomic DNA obtained from OSCC tissues for the status of exon 19 and 21 of EGFR as these regions have been shown to be frequently mutated in other cancers (Marchetti et al., 2005; Li et al., 2011; Peraldo-Neia et al., 2011; Teng et al., 2011). Direct sequencing analysis of the PCR amplicons revealed absence of mutation in any of the forty OSCC samples that were analyzed.

This observation in OSCC is in contrast to those that have been reported in NSCLCs, wherein 48% to 83% and 43% to 67% of tumor samples were found to carry exon 19 and 21 mutations respectively (Pao et al., 2004; Gazdar, 2009; Li et al., 2011). Mutations in these two exons have also been observed in triple negative breast cancer at a frequency of 1.4-11.4% (Weber et al., 2005; Generali, 2007; Teng et al., 2011) and at a frequency of 7.3% in laryngeal cancers (Lee et al., 2006). Investigations for aberrations in these two exons in other cancers such as tongue and tonsil squamous cell carcinomas, hepatocellular carcinoma, colon adenocarcinoma, gastric adenocarcinoma, ductal carcinoma of breast, acute adulthood leukemias, glioblastomas, bronchoalveolar and lymphoepithelioma-like carcinoma of lung, however, have indicated absence of mutations in them (Table 1).

Table 1. Incidence and Types of Mutations Reported in Tyrosine Kinase Domain Coding Exons of EGFR in Cancers

Type of cancer	Mutations and Incidence			Reference
	Exon 19	Exon 21	Other exons	
Oral cavity cancer	None	A859A (1.79%)	Exon 20 Q787Q (30.36%), G857R (3.56%), L862Q (3.56%)	Hsieh et al., 2011
Nasopharynx carcinoma	None	None	None	Soo-chin et al., 2006
Hepatocellular carcinoma	None	None	None	Soo-chin et al., 2006
Colon adenocarcinoma	None	None	None	Lee et al., 2004
Gastric adenocarcinoma	None	None	None	Lee et al., 2004
Breast ductal carcinoma	None	None	Exon 18 L707 (0.19%)	Lee et al., 2004
Hepatocellular carcinoma	None	None	None	Lee et al., 2004
Acute adulthood leukemia	None	None	None	Lee et al., 2004
Oral squamous cell carcinoma	None	None	Exon 18 T725T (0.58%)	Huang et al., 2009
Larynx cancer	K745R (1%)	None	None	Loeffler-Ragg et al., 2005
Colorectal cancer	None	None	Exon 18 G719S (0.34%)	Barber et al., 2004
Glioblastoma	None	None	None	Barber et al., 2004
Head and Neck squamous cell carcinoma	E746_A750 del (7.3%)	None	None	Lee et al., 2005
Gastric carcinoma	None	None	None	Lee et al., 2006
Colorectal carcinoma	None	None	None	Lee et al., 2006
Breast carcinoma	None	None	None	Lee et al., 2006
Broncioloalveolar carcinoma	E746_A750 del (36.36%) E746_T751 del (ins ala) (4.54%) L747_P753 del (ins ser) (4.54%) L747_P751 del (ins ser) (4.54%) L747_E749 del (4.54%)	L858M (4.54%) L858R (36.36%)	Exon 18 G719C (4.54%)	Marchetti et al., 2005
Conventional lung adenocarcinoma	E746_A750 del (23.53%) E746_T751 del (ins ala) (5.88%) L747_P753 del (ins ser) (5.88%)	L858R (52.94%)	Exon 18 G719C (11.76%)	Marchetti et al., 2005
Breast cancer	None	None	None	Bhargava et al., 2005
Lymphoepithelioma-like carcinoma of lung	None	None	None	Liu et al., 2013
Triple Negative Breast cancer	E746_A750 del (2.86%) S752_I759 del (2.86%)	L858R (1.43%), T847I (2.86%)	None	Hui-Fang Teng et al., 2011
Colorectal carcinoma	None	None	(Exon 20) M787I (22.41%)	Oh et al., 2011
Prostrate Cancer	T751I (1%), R748K (1%)	V851I (1%), G863D (1%), A839V (1%), L828M (1%), F856Y (1%), F856L (1%)	(Exon 20) E804G (1%), Q820R (1%), P872L (1%), F788L (1%), G796V (1%)	Peraldo-Neia et al., 2011

This together with difference in frequencies observed in different types of cancers as mentioned above suggests that the mutation events may be associated with the tissue of origin of the cancer. For example, the frequency of occurrence of mutations in the well known tumor suppressor gene, p53 has been shown to vary in many cancers (Olivier et al., 2010; Braakhuis et al., 2013). Based on this comparison we suggest that the absence of mutation in OSCC samples analyzed in the present study may be due to its tissue of origin. However, it is important to note that the tyrosine kinase domain of EGFR is coded by exons 18 to 21, and that mutations within exon 18 have indeed been observed, albeit at a lower frequency, in a few cancer types including ductal carcinoma of breast (Lee et al., 2004), colorectal carcinoma (Barber et al., 2004), bronchoalveolar carcinomas and conventional lung adenocarcinoma (Marchetti et al., 2005) (Table 1). Besides, a silent missense mutation T725T in exon 18 (Huang et al., 2009) and two missense mutations G857R and L862Q were also identified in exon 20 in patients with OSCC lesions from Taiwan (Hsieh et al., 2011). Hence it is possible that mutation within the other two exons – 18 and 20, may have occurred in the OSCC samples that were analyzed in the present study. However, it is important to note that the present study on OSCC was initiated based on high frequency of occurrence of mutations in exon 19 and 21 in NSCLCs that sensitizes the cancerous lesions to small molecule inhibitors of EGFR tyrosine kinase activity. Since the role of mutations in exon 18 and 20 in enhancing the sensitivity of cancerous lesions to small molecule inhibitors of EGFR tyrosine kinase activity is not known, the occurrence of mutations in them was not addressed.

Several studies have shown a positive association between the occurrence of exon 19 deletions and L858R missense mutation in exon 21 and sensitivity of small molecule inhibitors against EGFR tyrosine kinase activity (Gazdar et al., 2009). Unlike antibodies that block the ligand binding domain of EGFR, small molecule inhibitors such as erlotinib and gefitinib act by directly binding to the tyrosine kinase domain of EGFR (EGFR-TKD) to inhibit its tyrosine kinase activity (Gazdar et al., 2009).

The binding efficacy of the small molecule inhibitors with either exon 19 or 21 mutant EGFR-TKD is higher than that of wild type EGFR-TKD, and hence cancerous lesions carrying either one the above mutant EGFR-TKDs are relatively more sensitive to the inhibitors (Gazdar et al., 2009). The lack of mutation in exon 19 and 21 in OSCC samples analyzed in the present study, suggests that OSCC lesions may not show increased sensitivity to EGFR inhibitor drugs unlike NSCLCs. This data in association with earlier finding of lack of mutation in the c-Cbl binding domain of EGFR coded by exon 27 (Tushar and Ramanathan, 2013), infers that mutations in exons 19, 21 and 27 do not play a role in the genesis of OSCC. However, further research on other exons is essential to confirm the role of EGFR in the genesis of OSCC lesions, and evaluate their association with sensitivity towards EGFR tyrosine kinase inhibitors.

References

Asahina H, Yamazaki K, Kinoshita I, et al (2006). A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer*, **95**, 998-1004.

Barber TD, Vogelstein B, Kinzler KW et al (2004). Somatic mutations of EGFR in colorectal cancers and glioblastomas. *N Engl J Med*, **27**, 2283.

Benchekroun MT, Saintigny P, Thomas SM, et al (2010). Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. *Cancer Prev Res*, **3**, 800-9.

Bhargava R, Gerald WL, Li AR, et al (2005). EGFR gene amplification in breast cancer: correlation with epidermal growth factor receptor mRNA and protein expression and HER-2 status and absence of EGFR-activating mutations. *Mod Pathol*, **18**, 1027-33.

Braakhuis B, Rietbergen M, Buijze M, et al (2013). TP53 mutation and human papilloma virus status of oral squamous cell carcinomas in young adult patients. *Oral dis*, [Epub ahead of print].

Brand TM, Iida M, Li C, Wheeler DL (2011). The nuclear epidermal growth factor receptor signaling network and its role in cancer. *Discov Med*, **12**, 419-32.

D'Angelo SP, Pietanza MC, Johnson ML, et al (2011). Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol*, **29**, 2066-70.

Gazdar AF (2009). Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*, **28**, 24-31.

Generali D, Leek R, Fox SB, et al (2007). EGFR mutations in exons 18-21 in sporadic breast cancer. *Ann Oncol*, **18**, 203-5.

Hsieh CH, Chang JW, Hsieh JJ, et al (2011). Epidermal growth factor receptor mutations in patients with oral cavity cancer in a betelnut chewing-prevalent area. *Head Neck*, **33**, 1758-64.

Huang S, Chuang W, Chen I, et al (2009). EGFR protein overexpression and mutation in areca quid-associated oral cavity squamous cell carcinoma in Taiwan. *Head Neck*, **31**, 1068-77.

Huang Y, Chang Y (2011). "The Eighth Chapter" Prostate Cancer: From Bench to Bedside. Croatia, Intech publisher, 2011.

Inoue A, Suzuki T, Fukuhara T, et al (2006). Prospective phase ii study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol*, **24**, 3340-6.

Jayaraman B, Valiathan GM, Jayakumar K, et al (2012). Lack of mutation in p53 and H-ras genes in phenytoin induced gingival overgrowth suggests its non cancerous nature. A*sian Pac J Cancer Prev*, **13**, 5535-8.

Lee JW, Soung YH, Kim SY, et al (2004). Absence of EGFR mutation in the kinase domain in common human cancers besides non-small cell lung cancer. *Int J Cancer*, **113**, 510-11.

Lee JW, Soung YH, Seo SH, et al (2005). Carcinoma of the head and neck somatic mutations of egfr gene in squamous cell carcinoma of the head and neck. *Clin Cancer Res*, **11**, 2879-82.

Lee JW, Soung YH, Seo SH, et al (2006). Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. *Clin Cancer Res*, **12**, 57-61.

Li M, Zhang Q, Liu L, et al (2011). The different clinical significance of EGFR mutations in exon 19 and 21 in non-small cell lung cancer patients of China. *Neoplasma*, **58**, 74-81.

Li TJ, Ciu J (2013). COX-2, MMP-7 expression in oral lichen planus and oral squamous cell carcinoma. *Asian Pac J Trop Med*, **6**, 640-3.

Liu Q, Ma G, Yang H, et al (2013). Lack of epidermal growth factor receptor gene mutations in exons 19 and 21 in primary lymphoepithelioma-like carcinoma of the lung. *Thoracic Cancer*, **5**, 63-7.

Lo HW, Cao X, Zhu H, et al (2010). Cyclooxygenase-2 is a novel transcriptional target of the nuclear EGFR-STAT3 and EGFR VIII-STAT3 signaling axes. *Mol Cancer Res*, **8**, 232-45.

Loeffler-Ragg J, Witsch-Baumgartner M, Tzankov A, et al (2005). Low incidence of mutations in EGFR kinase domain in Caucasian patients with head and neck squamous cell carcinoma. *Eur J Cancer*, **42**, 109-11.

Lynch TJ, Bell DW, Sordella R, et al (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*, **350**, 2129-39.

Marchetti A, Martella C, Felicioni L, et al (2005). EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol*, **23**, 857-65.

Martín-Ezquerra G, Salgado R, Toll A, et al (2010). Multiple genetic copy number alterations in oral squamous cell carcinoma: study of MYC, TP53, CCDN1, EGFR and ERBB2 status in primary and metastatic tumours. *Br J Dermatol*, **163**, 1028-35.

Mitsudomi T, Yatabe Y (2009). Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *FEBS J*, **277**, 301-8.

Oh B, Lee R, Chung S, Ho Kim K (2011). Epidermal growth factor receptor mutations in colorectal cancer patients. *J Korean Soc Coloproctol*, **27**, 127-32.

Olivier M, Hollstein M, Hainaut P (2010). TP53 mutations in human cancers: Origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*, **2**, 001008.

Paez JG, Jänne PA, Lee JC, et al (2004). EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*, **304**, 1497-500.

Pao W, Miller V, Zakowski M, et al (2004). EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA*, **101**, 13306-11.

Pennock S, Wang Z (2008). A tale of two Cbls: interplay of c-Cbl and Cbl-b in epidermal growth factor receptor downregulation. *Mol Cell Biol*, **28**, 3020-37.

Peraldo-Neia C, Migliardi G, Mello-Grand M, et al (2011). Epidermal Growth Factor Receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in primary prostate cancer. *BMC Cancer*, **11**, 31.

Rajendran S, Muthupalani RS, Ramanathan A (2013). Lack of RING finger domain (RFD) mutations of the c-cbl gene in oral squamous cell carcinomas in Chennai, India. *Asian Pac J Cancer Prev*, **14**, 1073-5.

Sasaki H, Okuda K, Endo K, et al (2007). CCND1 messenger RNA expression is correlated with EGFR mutation status in lung cancer. *Clin Lung Cancer*, **8**, 493-6.

Shintani S, Li C, Mihara M, et al (2004). Gefitinib ('Iressa', ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, up-regulates p27KIP1 and induces G1 arrest in oral squamous cell carcinoma cell lines. *Oral Oncol*, **40**, 43-51.

Soo-Chin L, Seng-Gee L, Ross S (2006). Lack of somatic mutations in EGFR tyrosine kinase domain in hepatocellular and nasopharyngeal carcinoma. *Pharmacogenetics Genomics*, **16**, 73-4.

Teng YH, Tan WJ, Thike A, et al (2011). Mutations in the epidermal growth factor receptor (EGFR) gene in triple negative breast cancer: possible implications for targeted therapy. *Breast Cancer Res*, **13**, 35.

Tushar MD, Ramanathan A (2013). Tyrosine 1045 codon mutations in exon 27 of EGFR are infrequent in oral squamous cell carcinomas. *Asian Pac J Cancer Prev*, **14**, 4279-82.

Valiathan GM, Thenumgal SJ, Jayaraman B, et al (2012). Common docking domain mutation E322K of the ERK2 gene is infrequent in oral squamous cell carcinomas. *Asian Pac J Cancer Prev*, **13**, 6155-7.

Van Damme N, Deron P, Van Roy N, et al (2010). Epidermal growth factor receptor and K-RAS status in two cohorts of squamous cell carcinomas. *BMC Cancer*, **10**, 189.

Woelk T, Sigismund S, Penengo L, Polo S (2007). The ubiquitination code: a signalling problem. *Cell Div*, **2**, 11.

Weber F, Fukino K, Sawada T, et al (2005). Variability in organ-specific EGFR mutational spectra in tumour epithelium and stroma may be the biological basis for differential responses to tyrosine kinase inhibitors. *Br J Cancer*, **92**, 1922-6.

Zanaruddin SNS, San Yee P, Yii Hor S, et al (2013). Common oncogenic mutations are infrequent in oral squamous cell carcinoma of Asian origin. *PLoS ONE*, **8**, 80229.