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Low incidence of mutations in *EGFR* kinase domain in Caucasian patients with head and neck squamous cell carcinoma

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ABSTRACT

Somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene are associated with increased sensitivity to tyrosine kinase inhibitors (TKIs) and are present in 10–30% of non-small cell lung carcinoma depending on ethnic origin. EGFR protein is also overexpressed in about 90% of squamous cell carcinoma of head and neck (HNSCC), and treatment with TKIs has shown clinical benefit in a subgroup of these patients. Recently, *EGFR* mutations were described in three Asian patients with larynx cancer. We screened for *EGFR* tyrosine kinase mutations in tumour DNA of 100 patients of Caucasian origin with HNSCC by direct sequencing of the hotspot regions. Only one patient with larynx cancer displayed a novel, somatic *EGFR* missense mutation, K745R, affecting a highly conserved residue within the ATP cleft. Similar to reports in lung cancer, *EGFR* kinase domain mutations in HNSCC patients seem to show a lower incidence in patients of Caucasian origin.

1. Introduction

Aberrant expression of epidermal growth factor receptor (EGFR) is involved in signalling pathways responsible for proliferation, survival, invasion, angiogenesis and metastasis in many cancers including head and neck squamous cell carcinoma [1–3]. Strategies in cancer therapy aimed at interrupting this signalling pathway include monoclonal antibodies or small-molecule tyrosine kinase inhibitors (TKIs) such as gefitinib (ZD1839, Iressa®) or erlotinib (Tarveca®) [4,5]. The impact of EGFR expression levels on drug sensitivity to EGFR blockers is still debatable, since preclinical studies and clinical data have shown no correlation between EGFR expression and response [6–10]. Mutations in the *EGFR* tyrosine kinase domain seem to correlate with clinical response in patients with non-small cell lung cancer [11,12]. All these *EGFR* mutations affect amino acids near the ATP-binding pocket that is targeted by TKIs. Of these mutations, 86% cluster in two hotspots, in exon 19 and 21. The remaining 14% are rare and scatter throughout exons 18–21 [13].

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Recently, the in-frame deletion mutation E746-A750del in exon 19 was found in three out of 41 Korean patients with HNSCC [14].

To explore the frequency of specific somatic *EGFR* tyrosine kinase mutations in Caucasian patients with HNSCC, we sequenced 100 cancer samples from patients with advanced primary or relapsed HNSCC. Only one tumour demonstrated a mutation in the ATP-binding pocket suggesting that *EGFR* mutations in HNSCC are less frequent, depending on ethnic origin.

2. Materials and methods

2.1. Patient samples

With the approval of the local institutional review board 138 formalin-fixed paraffin-embedded HNSCC samples were selected from the local tumour bank covering cases diagnosed between 1992 and 2004. All samples were obtained from Caucasian patients and analysed independently by two pathologists (routine diagnostician and AT). A total of 134 patients were diagnosed with squamous cell carcinoma, two with adenoid-cystic (cyclindromas) and two with Schmincke lymphoepithelioma. Six patients received TKI gefitinib during the later course of disease. The male to female ratio was 4.9:1 and patient age ranged from 21 to 97 years (median age 56.5 ± 2.1).

For subsequent DNA isolation tumour-cell rich areas with a tumour-cell proportion exceeding the stroma component by >90% were selected on haematoxylin- and eosin-stained slides. For DNA extraction, tissue cores (cylinders) with a diameter of 1.5 mm and a depth of 5 mm were punched from these areas using a TrapSystem biopsy needle (Medical Devise Techn., Inc., Gainesville, FL, USA). Normal tissues or precancerous lesions were investigated only in patients carrying the mutation.

2.2. DNA extraction and EGFR gene analysis

DNA was extracted using the BioRobot M48 workstation with MagAttract technology (Qiagen, Germany). PCR of exons 18, 19, and 21 of the *EGFR* gene was performed with primers described previously [12]. The hotspot regions in exon 19 and 21 [13] were analyzed in all patients. In addition, to extend the mutation detection rate [13] exon 18 was analyzed in three patients responding to treatment with TKIs. Sequencing of PCR products was carried out using a Big Dye sequencing kit (Perkin–Elmer, Foster City, CA) on the ABI Genetic Analyzer 3100.

3. Results

High-quality tumour DNA for PCR analysis was extracted in only 100 of the 138 HNSCC samples due to degraded DNA in paraffin-embedded tissues [15]. The tumours included for further analysis consisted of 37 oral cavity cancer samples (including nine mouth floor, two tongue and six tonsillar cancers), 39 larynx, 15 hypopharynx, and nine nasopharynx carcinoma (containing two lymphoepithelial and one adenoid-cystic carcinoma).

Direct sequencing of exon 19 of the *EGFR* gene revealed a mutation at the ATP binding site in only one patient. This mutation with a heterozygous A to G change at nucleotide 2232 has not been shown to date and results in a lysine to arginine substitution at codon 745 (K745R) (Fig. 1).

The affected patient (ZF, 72 years) suffered from laryngeal carcinoma arising in the left vocal cord and was treated by laryngectomy. Six non-tumourous samples from the patient (soft tissue, lymph nodes, unaffected squamous epithelium) displayed wildtype sequence for position 745, indicating that the mutation had developed somatically. Furthermore, three endoscopic biopsies from carcinoma in situ at different laryngeal sites did not show the mutation. Experiments were repeated twice to ensure specificity of results, including the repetition of PCR amplification and reverse sequencing.

We also found two novel single nucleotide polymorphisms (SNPs) in intron 19: IVS19+69GA with 0.03 and IVS19+96AG with 0.27 allele frequency in the somatic tissue.

The patient with the K745R mutation also displayed the SNP IVS19+69GA in the invasive cancer sample, but only in one of three carcinoma in situ samples (collected from the same left site as the invasive tumour), and in none of the six normal tissue controls. These data show that the SNP IVS19+69GA also developed somatically.

Direct sequencing of exon 21 of the *EGFR* gene in tumour tissues of 100 patients revealed no mutation. In this exon, the SNP R836R occurred with 0.016 allele frequency. This polymorphism has already been described in control population with a frequency of 0.028 (http://www.ncbi.nlm.nih.gov/SNP; SNP ID rs17518376).

Three HNSCC patients who showed clinical response after therapy with gefitinib displayed no *EGFR* mutations in the sequenced exons.

4. Discussion

In non-small cell lung cancer *EGFR* kinase domain mutations are known to increase the response rate to oral EGFR TKIs.

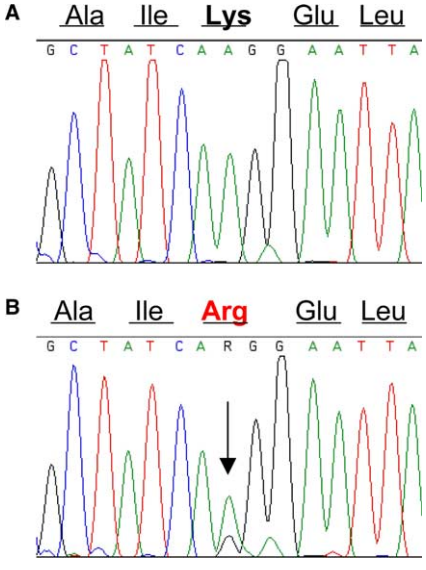

Fig. 1 – *EGFR mutation K745R in exon 19*. (A) The wildtype nucleotide sequence (forward sequence). (B) The heterozygous missense mutation with A to G substitution of nucleotide 2232 results in a lysine to arginine substitution at codon 745 (K745R).

They cluster near the ATP cleft of the tyrosine kinase domain and mediate binding of compounds such as gefitinib or erlotinib [13]. These somatic mutations occur predominantly in adenocarcinomas of the lung, preferentially in women, never-smokers, and patients of East Asian origin, but have rarely been reported in other human cancers [16]. Gefitinib also displays anticancer activity in head and neck cancer [17], but predictors of sensitivity are not yet characterized. Recently, Lee et al. [14] described the presence of *EGFR* mutations in three of 41 patients with HNSCC. In contrast to the frequency of *EGFR* mutations observed in Korean patients (7.3%), in our study only one patient with larynx cancer (1%) harboured a mutation within the *EGFR* tyrosine kinase domain. These data suggest an ethnic difference in the frequency of *EGFR* mutations in HNSCC that is also seen in lung cancer [13]. In non-small cell lung cancer *EGFR* mutations were more frequent in patients of East Asian origin than in Caucasian patients. As the aetiology of *EGFR* mutations is unknown, epidemiologic studies of Asian and Caucasian patients may provide some clues.

The mutation K745R found in our patient with larynx cancer has not been described previously. Matched normal tissue showed the wildtype sequence, indicating that the mutation arose somatically during tumour formation, and probably even during progression to invasive cancer, taking into consideration the lack of mutation in carcinoma in situ samples.

The pathogenicity of this mutation is supported by the observation that lysine at position 745 is conserved among all human tyrosine kinases with similar ATP-binding domain, suggesting that this residue is critical for the protein structure and function [18–21].

The hotspot in-frame exon 19 deletions observed in lung cancer occur just downstream from this lysine residue at position K745 [12]. It is postulated that these mutations result in repositioning of critical residues, which stabilizes their interaction with both ATP and its competitive inhibitor gefitinib [12].

In summary, mutations in the ATP-binding domain of the *EGFR* kinase are rare events in patients of Caucasian origin with HNSCC. However, for individualized cancer therapy screening for such specific mutations could be one rationale for clinical applicability of gefitinib to a subset of patients.

Conflict of interest statement

None declared.

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