Epidermal growth factor receptor kinase domain mutations are rare in salivary gland carcinomas

Malignant salivary gland neoplasms account for <0.5% of all malignancies and approximately 3–5% of all head-and-neck cancers (Milano et al, 2007). Salivary gland carcinomas (SGCs) represent a histopathologically heterogeneous group with different biological behaviour and clinical outcome. The main histopathologic types are (1) mucoepidermoid carcinoma that represents 29–34% of malignant tumours; (2) adenoid cystic carcinoma (ACC; 20%); (3) adenocarcinoma including acinic cell carcinoma; and (4) salivary duct carcinoma (Milano et al, 2007). Progress in understanding the cell biology of SGCs and detecting vulnerable molecular pathways may lead to the development of new targeted therapy options. Epidermal growth factor receptor (EGFR; also known as ErbB1) is considered a possible key pathway for therapeutic intervention, because activating EGFR mutations are associated with improved clinical response to tyrosine kinase inhibitor therapy. Recently, we identified two EGFR mutations in a cohort of 25 salivary gland carcinomas (SGCs) by screening the tumour samples for the both most common hotspot mutations in exons 19 and 21 by allele-specific PCR. Here, we present a comprehensive sequencing analysis of the entire critical EGFR tyrosine kinase domain in 65 SGC of the main histopathological types. We found EGFR mutations in the tyrosine kinase domain to be a rare event in SGCs. No additional mutations other than the two known exon 19 deletions (c.2235_2249del15) in a mucoepidermoid carcinoma and an adenoid cystic carcinoma have been detected. Other putative predictive markers for EGFR-targeted therapy in SGCs might be relevant and should be investigated.

Keywords: epidermal growth factor receptor; mutation screening; salivary gland carcinomas; targeted therapy

MATERIALS AND METHODS

Surgically removed formalin-fixed tumour samples were obtained from 65 patients (35 males and 30 females with a median age at diagnosis of 55 years) treated with the histopathological diagnosis of an SGC according to the WHO classification (Barnes et al, 2005). All patients received surgery and in selected cases of postoperative radiation therapy. Epidermal growth factor receptor-targeted therapy was not applied. The study cohort consisted of ACC (n = 25), mucoepidermoid carcinoma (n = 10), myoepithelial carcinoma (n = 8), acinic cell carcinoma (n = 12) and adenocarcinoma ex pleomorphic adenoma (n = 10).

Deoxyribonucleic acid was isolated from microdissected tumour areas using the QiAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany).

Epidermal growth factor exons 18, 19, 20 and 21 were amplified by nested PCR using primers described previously (Eberhard et al, 2005). Polymerase chain reaction products were purified with the QIAquick PCR Purification Kit (Qiagen GmbH). Genomic sequencing was performed using fluorescent dye-terminator chemistry and the primer M13F: 5'-TGTAAAACGACGGCCAGT-3' (MWG Biotech, Martinsried, Germany). The reported mutations were scored as reproducible, having been identified in two independent PCR amplifications and by M13 sequencing in both directions (M13R: 5'-CAGGAAACACGTATGACC-3').

The numbering of the EGFR nucleotide and amino acid positions was according RefSeq sequences NM 005228.3 and NP 005219.2, respectively.

This study was conducted in accordance with the principles of the Declaration of Helsinki, as adopted by the 29th World Medical Assembly, Helsinki, Finland.

RESULTS

Sequencing analysis of exons 18–21 of the EGFR tyrosine kinase domain in 65 SGCs revealed two cases (3.1, 95% exact confidence limit: 0.5–1.2) with a heterozygous exon 19 deletion mutation. The two exon 19 E746_A750 deletions (both of them were variant c.2235_2249del15) were identified in a mucoepidermoid carcinoma of the left parotid gland grade 2 (54-year-old female patient, non-smoker), and in an ACC of solid type of the right parotid gland (69-year-old male patient, non-smoker). The female patient with ACC grade 2 (54-year-old female patient, non-smoker), and in an ACC of solid type of the right parotid gland grade 2 (54-year-old female patient, non-smoker), and in an ACC of solid type of the right parotid gland grade 2 (54-year-old female patient, non-smoker).

In conclusion, drug-sensitising EGFR mutations are rather rare in SGCs and seem to be mainly found in a few mucoepidermoid and ACC. Taking into consideration the encouraging relative high number of responding SGC patients (particularly ACC patients with poor prognosis) who achieved stable disease after cetuximab therapy with gefitinib administered in a genotype-directed fashion in two recent trials in advanced non-small cell lung cancer, therapy with gefitinib administered in a genotype-directed fashion in patients harbouring EGFR mutations resulted in very favourable clinical outcomes with good tolerance (Sequist et al, 2008; Tamura et al, 2008).

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