Oral Oncology 49 (2013) 576–581

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Frequency of *Fibroblast Growth Factor Receptor 1* gene amplification in oral tongue squamous cell carcinomas and associations with clinical features and patient outcome

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ARTICLE INFO

Article history:
Received 24 September 2012
Received in revised form 13 January 2013
Accepted 19 January 2013
Available online 19 February 2013

Keywords:
Fibroblast Growth Factor Receptor 1
FGFR1
Oral tongue squamous cell carcinoma
Oral cancer
Fluorescence *in situ* hybridisation
FISH
Gene amplification
Outcome

SUMMARY

Objectives: Novel therapies are required for patients with recurrent or metastatic oral tongue squamous cell carcinoma (OTSCC). *Fibroblast Growth Factor Receptor 1* (*FGFR1*) amplification frequently occurs in squamous cell carcinoma of the lung and represents a novel druggable therapeutic target in this and other malignancies. This study examined the frequency and clinical associations of *FGFR1* amplification in OTSCC.

Materials and methods: The frequency of *FGFR1* amplification determined by fluorescence *in situ* hybridization was evaluated in a cohort of 123 OTSCC patients. Associations of *FGFR1* amplification with clinical characteristics and outcome were determined.

Results: *FGFR1* gene amplification was present in 9.3% (10/107) of cases and was significantly associated with smoking status ($P = 0.03$). *FGFR1* amplification was seen more commonly in males (9/10 amplified cases male, $P = 0.16$) and there were no associations with age, stage, T stage, nodal status, alcohol history or performance status (all $P > 0.05$). Outcome was not significantly different between *FGFR1* amplified and non-amplified patients.

Conclusions: Copy number variations of the *FGFR1* gene occur in a subset of OTSCC with approximately 10% of cases showing amplification of the gene. *FGFR1* amplification may represent a therapeutic target in OTSCC.

Introduction

Carcinomas of the oral cavity have a poorer prognosis compared with other head and neck subsites.[1] Furthermore, within the oral cavity, oral tongue squamous cell carcinomas (OTSCC) have the worst prognosis.[2] Early stage OTSCC are typically managed with surgery with curative intent, however there remains a significant rate of local locoregional failure such that the overall outcome for early stage OTSCC is inferior to outcomes for later stage cancers of other oral cavity sites.[2] While HPV status is now known to play a causative role in a significant proportion of head and neck squamous cell carcinoma (HNSCC) patients in the oropharynx,[3] the involvement of HPV in OTSCC remains unclear, though most studies suggest that HPV infection is not significant in the pathogenesis of OTSCC.[4,5] The lack of a pathological explanation for the aggressive tumour biology of OTSCC, and limitations of traditional clinico-pathological criteria to appropriately stratify patients for particular treatments, suggest that further research is needed to identify prognostic and predictive biomarkers for existing therapies and also to identify novel molecular targets of therapeutic utility in OTSCC.

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http://dx.doi.org/10.1016/j.oraloncology.2013.01.006

The Fibroblast Growth Factor (FGF) family consists of at least 23 secreted glycoproteins that bind to and signal through four highly conserved trans-membrane receptor tyrosine kinases, FGF receptors 1–4.[6,7] Normally, FGF signalling regulates many developmental pathways and physiological processes such as proliferation, differentiation and survival in a variety of cell types.[8] There is strong evidence that deregulated FGF signalling plays a role in the pathogenesis of many cancers, and in particular, evidence of an oncogenic role for *FGFR1*. Several recent studies have investigated *FGFR1* gene amplification in squamous cell carcinoma (SCC) of the lung.[9–12] Weiss et al.[9] found frequent and focal *FGFR1* amplification in 22% of cases, a finding confirmed by a subsequent study reporting *FGFR1* amplification in 20% of pulmonary squamous carcinomas.[10] Dutt et al.[11] independently found an *FGFR1* amplification rate of 21% in lung SCC using SNP arrays. Kim et al.[12] found an amplification rate of 13% in surgically resected SCC of the lung. Significantly, *in vitro* data indicate that *FGFR1* amplified lung SCC cells are dependent on *FGFR1* signalling for survival and are sensitive to *FGFR* inhibitors.[9,11] Less frequent amplifications in the *FGFR1* gene have also been described in several other cancer types including breast,[13] oesophageal,[14] bladder,[15] prostate[16] and ovarian.[17] In 2007, Freier et al.[18] demonstrated that 17.4% of oral cavity squamous cell carcinomas harboured amplifications of *FGFR1* suggesting that this may be a relevant therapeutic target in this malignancy.

The potential to exploit *FGFR1* as a therapeutic target has led to the development of several potent and specific *FGFR1* inhibitors, which are at various stages of preclinical and early clinical development.[6] In the present study we have utilised a fluorescence *in situ* hybridisation (FISH) assay to investigate the frequency of *FGFR1* gene amplification in a cohort of 123 OTSCC patients, and examined the associations of *FGFR1* gene copy number variations with clinical features and patient outcome.

Table 1
Associations of *FGFR1* amplification status with patient clinical characteristics.

Characteristic	All	*FGFR1* amplified	*FGFR1* non-amplified	*P* value
Total number	123	10	97	
Sex				
Male	80 (65)	9 (90)	63 (65)	0.16
Female	43 (35)	1 (10)	34 (35)	
Age at diagnosis; years	59	57.5	56.0	
Median (range)	(21–93)	(42–74)	(21–87)	0.96
Stage				
I	34 (28)	2 (20)	25 (26)	0.40
II	36 (30)	3 (30)	31 (32)	
III	13 (11)	0	12 (13)	
IV	39 (32)	5 (50)	28 (29)	
T stage				
1	40 (33)	3 (30)	29 (30)	0.96
2	47 (39)	4 (40)	39 (41)	
3	13 (11)	1 (10)	11 (11)	
4	22 (18)	2 (20)	17 (18)	
N stage				
0	79 (64)	5 (50)	64 (66)	0.34
1	11 (9)	1 (10)	8 (8)	
2	33 (27)	4 (40)	25 (26)	
Smoking history				
No/never	34 (29)	0	31 (33)	0.03*
Yes	85 (71)	10 (100)	64 (67)	
ETOH history; >20 g/day				
No/never/social	74 (62)	4 (40)	60 (64)	0.18
Yes	45 (38)	6 (60)	34 (36)	
ECOG performance status				
0	71 (59)	6 (60)	55 (58)	0.78
1	38 (31)	4 (40)	30 (32)	
2	8 (7)	0	8 (8)	
3	4 (3)	0	2 (2)	

Note: Total numbers of patients in each category vary as some data was not available. All figures are number (%). *P* values are for the comparison of the *FGFR1* gene amplification and non-amplification groups.
* Indicates a statistically significant association.

Patients and methods

Patients

The study comprised a cohort of 123 patients treated for oral tongue squamous cell carcinoma at our institution between 2002 and 2008. OTSCC was defined by a multidisciplinary team as a tumour arising from the anterior two-thirds of the tongue. The clinical patient and tumour characteristics are summarised in Table 1. Patients with a history of tobacco use were defined by a clinician as an "ever-smoker" or a "non-smoker". A history of alcohol use was defined as greater than 20 g of alcohol per day. The Institutional Ethics Committees approved the study protocol. All tissue samples used for the FISH studies described below were taken prior to the commencement of treatment.

Tissue microarrays

Formalin fixed paraffin embedded (FFPE) tumour tissue blocks were obtained for each of the 123 patients. A pathologist identified an area containing the most tumour on a haematoxylin and eosin stained section from each FFPE tumour block. Representative 1 mm tumour cores were punched from this area for creation of tissue microarray (TMA) blocks. Sections were then cut from each TMA block for FISH assay.

FGFR1 fluorescence in situ hybridisation

Four micron FFPE tissue sections from each TMA block, or whole tissue sections from the original FFPE block in the case of missing TMA cores, were incubated at 60 °C for 1 h to melt paraffin, then further deparaffinised in xylene and rehydrated through graded alcohols to water. Target retrieval was performed in Heat Pretreatment Solution (Invitrogen) in a pressure cooker at 125 °C for 2½ min followed by three short water washes. Sections were treated with Enzyme Pretreatment Reagent (Invitrogen) for 15 min at 37 °C. Slides were dehydrated through 70%, 80%, 90% and 100% alcohol solutions and air dried. *FGFR1*/CEN8 probe mix (Abnova) was added to the slide which was coverslipped and sealed with rubber cement to prevent evaporation during hybridisation. Slides were denatured for 5 min at 80 °C then hybridised for 18 h at 37 °C on a StatSpin Hybridiser (Dako). After hybridisation the coverslips were removed and slides washed in two solutions of a 0.5× SSC stringent wash buffer; for 2 min at room temperature followed by 5 min at 75 °C. After three washes in water sections were mounted in Vectashield mounting medium with Dapi (Vector), coverslipped and stored at 4 °C in the dark prior to being scored, typically within 48 h. The *FGFR1* probe, labelled with Texas Red, hybridises to the 8p12 region of chromosome 8, which includes the *FGFR1* gene. The CEN8 (chromosome 8) probe, labelled with FITC, hybridises to the 8q11.21 region.

Scoring of *FGFR1* FISH was performed on an Olympus BX51 fluorescence microscope. For each slide, individual cores or predefined areas of tumour were identified at low (×10) magnification using the DAPI filter. A minimum of 50 cells, typically 15–20 from each of three fields, were scored for the number of *FGFR1* (red) and chromosome 8 (green) signals using a 100× oil immersion lens. Raw data was entered into a Microsoft Excel spreadsheet for calculation