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Tendencies of FGFR2 rs2981582 polymorphism in patients with oral cancer

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SUMMARY

Objective. Fibroblast growth factor receptor 2 (FGFR2) is a member of the FGFR family of tyrosine kinase receptors, which via cell growth, invasiveness, motility and angiogenesis contributes to the process of tumorogenesis. A huge interest today is focused on FGFR2 gene polymorphism as it may have a significant impact on the development of various benign and malignant tumors.

A case-control study was designed to help determine if FGFR2 gene polymorphism rs2981582 is associated with oral cancer in Lithuanian subjects.

Methods. The study included 35 patients with a diagnosis of oral cancer and 100 healthy subjects as a reference group. DNA samples were extracted from peripheral venous blood. Genotyping of FGFR2 rs2981582 was performed using the real-time polymerase chain reaction method. Statistical analysis was performed using „IBM SPSS Statistics 20.0".

Results. It was determined that FGFR2 gene rs2981582 polymorphism has no effect on a development of oral cancer. The analysis of FGFR2 gene polymorphisms did not reveal any differences in the distribution of GG, GA, and AA genotypes between the oral cancer group, and the control group (42.9%, 48.6%, and 8.6% vs. 46%, 37% and 17%, respectively).

Conclusion. Results of present study showed no association between FGFR2 gene polymorphisms rs2981582 and oral cancer. However, a further study with a larger sample sizes is advisable.

Keywords: fibroblast growth factor receptor 2, gene polymorphism, oral cancer.

INTRODUCTION

Oral cancer (OC), also known as mouth cancer, is a part of a cancers group commonly referred to as head and neck cancers, and is considered as any cancerous tissue growth located in the oral cavity (1). Oral cancer causes more deaths than any other oral disease (2). It may appear as a primary lesion in any of the tissues in the mouth by metastasis from a distant site of origin or by extension from a neighboring anatomic structure, such as the nasal cavity. There are several types of oral cancers, but around 90% of them are squamous cell carcinomas, originating in the tissues that line the mouth and lips. Oral or mouth cancer includes cancers of lips, cheeks, floor of the mouth, hard and soft palate, paranasal sinuses, and pharynx. However, the most commonly detected is tongue cancer.

Carcinogenesis is a multi-step process including aberrant expression of two interacting classes of genes – oncogenes and tumour suppressor genes. Advanced oral cancer stages demonstrate cumulative molecular aberrations, with greater than 95% samples showing oncogene involvement (3). Several factors including angiogenesis, lymphangiogenesis, alterations in expression or structure of tumor suppressor genes, oncogenes and their proteins are involved in malignant transformation of potentially malignant oral lesions to oral carcinoma (2, 3).

Cell proliferation and differentiation during development and tissue repair is regulated by the fibroblast growth factor (FGF) receptor. FGF receptor family consists of four members (FGFR-1 (flg), FGFR-2 (bek), FGFR-3 and FGFR-4) that have 55-72% amino acid homology (4). The tumor derived FGF-2 may

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promote cancer progression by elevating proteolytic enzymes or by paracrine stimulation of vascular endothelial cell growth (5). The FGF family made up a large family of more than 20 members all of which retain specificities for both different FGFR family members and different isoforms of each receptor (6). Aberrant FGFR signaling has been implicated in the development of multiple cancer types (7, 8).

FGF-2 plays an important role in a regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. Also it can induce angiogenesis (9-11) and its receptors are important in synthesis of collagen. FGF-2 is involved in the transmission of signals between the epithelium and connective tissue, and influences growth and differentiation of a wide variety of tissue including epithelia (12). Studies have reported that FGF-2 manifests with overexpression in high grade malignant tumours and malignant transformation of normal cells transfected with FGF-2 gene (13). Invasion of cancer cells and proliferation of fibroblasts around cancer cells in an autocrine or paracrine fashion is one more function of FGF-2 (14). However, the results of few studies on expression of this factor in head and neck carcinomas are highly controversial (15-18).

Therefore, the aim of the present study is to determine the association of FGFR2 rs2981582 single nucleotide polymorphism (SNP) with oral carcinoma in investigated patients.

METHODS

All the procedures used in this study were approved by the Kaunas Regional Ethics Committee for Biomedical Research, Lithuania in compliance with ethical standards (permission number is BE-2-34). The study was conducted at the Department of Otorhinolaryngology, and Ophthalmology laboratory of Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Study population composition

The current study included 35 patients with oral cancer and 100 subjects as a control group. Subjects who were chosen to participate as a control group did not get any treatment previously or demonstrated any pathology on the examination day (Table 1).

Patients of the control group included 75% of males and 25% of females. There were no statistically significant gender or age differences between patients and control (Table 1). According to the presented data, 94.3% of patients with oral cancer had a history of smoking. Stage 2 oral cancer was tended to be diagnosed more frequently then cancer of stage 3 or stage 4 (Table 1).

Otorhinolaringological evaluation

Otorhinolaringological and general-medical examination was carried out as the procedures described elsewhere (19).

DNA extraction and genotyping

For DNA extraction, blood samples were collected from each individual in ethylenediaminetetraacetic

Table 1. Characteristics of study groups

	Oral cancer (n=35)	Control group (n=100)	*p* value
Males n (%)	26 (74.3)	75 (75)	0.549
Females n (%)	9 (25.7)	25 (25)	
Age, min/med/max	27/56/88	26/54.5/56	0.417
Smoking n (%)	33 (94.3)	–	–
Tumor differentiation grade G n (%)			
1	–		
2	22 (62.9)	–	–
3	11 (31.4)		
4	2 (5.7)		
Stage			
1	–		
2	11 (31.4)	–	–
3	12 (34.3)		
4	12 (34.3)		

Table 2. Frequency of FGFR2 rs2981582 genotype in the patients with oral cancer and the control group

Genotype/alelle	Oral cancer (n=35)	P HWE	Control group (n=100)	P HWE	*p* value
GG	15 (42.9)		46 (46.0)		
GA	17 (48.6)	0.551	37 (37.0)	0.055	0.338
AA	3 (8.6)		17 (17.0)		
G	47 (67.1)		129 (64.5)		0.690
A	23 (32.9)		71 (35.5)		

HWE – Hardy-Weinberg equilibrium.

Table 3. Binomial logistic regression analysis in the patients with oral cancer and the control group

Model	Genotype	95 % CI	*p* value
Codominant	GA vs. GG	1.409 (0.622-3.193)	0.411
	AA vs. GG	0.541 (0.139-2.103)	0.376
Dominant	GA + AA vs. GG	1.136 (0.523-2.469)	0.748
Recessive	AA vs. GG + GA	0.458 (0.126-1.668)	0.236
Overdominant	GA vs. GG + AA	1.608 (0.739-3.499)	0.231
Additive	A	0.900 (0.521-1.554)	0.706

(EDTA) tubes during their health examination. The DNA extraction and analysis of the gene polymorphism of FGFR2 rs2981582 was carried out at the Laboratory of Ophthalmology, Institute of Neuroscience, LUHS. DNA was extracted from white blood cells using the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations. DNA for the analysis of the FGFR2 rs2981582 gene polymorphisms was extracted from venous blood white blood cells using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA Kit, Thermo Scientific), according to the manufacturer's recommendations. DNA aliquots were stored at -20ºC until analysis.

Genotyping was carried out using the real-time polymerase chain reaction (RT-PCR) method. FGFR2 rs2981582 SNP were determined using TaqMan® SNP Genotyping assays (Applied Biosystems, Foster City, CA, USA) and their genotyping performed using a Rotor – Gene Q real-time PCR quantification system (Qiagen, USA). Thermal cycling conditions for PCR were, first, denaturation at 95°C for 10 min, followed by 45 cycles of 92°C for 15 s and 60°C for 1 min. 30 s. The Allelic Discrimination software (Qiagen, USA) was used to determine the individual genotypes, according to the fluorescence intensity rate of different detectors (VIC and FAM).

Table 4. Frequency of FGFR2 rs2981582 genotype in the patients with oral cancer and in the control groups by gender

Genotype/alelle	Males		P value	Females		*p* value
	Oral cancer (n=26)	Control group (n=75)		Oral c ancer (n=9)	Control group (n=25)	
GG	11 (44.4)	34 (54.3)	0.661	4 (44.4)	12 (48.0)	0.355
GA	12 (46.2)	28 (37.3)		5 (55.6)	9 (36.0)	
AA	3 (11.5)	13 (17.3)		0 (0)	4 (16.0)	
G	34 (65.4)	96 (64.0)	0.857	13 (72.2)	33 (66.0)	0.628
A	23 (32.9)					

Table 5. Binomial logistic regression analysis in the patients with oral cancer and the control group by gender

Model	Genotype	95 % CI	*p* value
Females			
Codominant	GA vs. GG	1.667 (0.346-8.038)	0.525
	AA vs. GG	-	0.999
Dominant	GA + AA vs. GG	1.154 (0.250-5.335)	0.855
Recessive	AA vs. GG + GA	-	0.999
Overdominant	GA vs. GG + AA	2.222 (0.473-10.447)	0.312
Additive	A	0.758 (0.238-2.415)	0.639
Males			
Codominant	GA vs. GG	1.325 (0.508-3.456)	0.566
	AA vs. GG	0.713 (0.171-2.974)	0.643
Dominant	GA + AA vs. GG	1.131 (0.459-2.784)	0.789
Recessive	AA vs. GG + GA	0.622 (0.162-2.384)	0.489
Overdominant	GA vs. GG + AA	1.439 (0.584-3.546)	0.429
Additive	A	0.948 (0.510-1.764)	0.866

Table 6. Frequency of FGFR2 rs2981582 genotype across the stages of cancer

Genotype	Stages of cancer			χ^2	*p* value
	2n (%)	3n (%)	4n (%)		
GG (n=15)	3 (20.0)	6 (40.0)	6 (40.0)	3.327	0.505
Male	105 (40.2%)	37 (35.2)	43 (41.0%)	25 (23.8%)	
Total	261	88	114	59	

Table 7. Genotype frequency across the cancer grades (G)

Genotype	Cancer grade (G)			χ^2	*p* value
	G2 n (%)	G3 n (%)	G4 n (%)		
GG (n=15)	9 (60.0)	4 (26.7)	2 (13.3)	4.954	0.292
GA (n=17)	10 (58.8)	7 (41.2)	0 (0)		
AA (n=3)	3 (100)	0 (0)	0 (0)		

Statistical analysis

Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). The data are presented as absolute numbers with percentages in brackets and average of age. The frequencies of genotypes and alleles (in percentage) are presented in Table 2.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of polymorphism rs2981582 using the χ^2 test in all groups. The distribution of the FGFR2 rs2981582 single-nucleotide polymorphism (SNP) in the oral carcinoma and control groups was compared using the χ^2 test or the Fisher exact test. Risk prediction for the patients with oral carcinoma of the floor of the mouth with FGFR2 rs2981582 gene polymorphism was calculated by logistic regression analysis. Differences were considered statistically significant when $p<0.05$.

RESULTS

Genotyping analysis showed that FGFR2 rs2981582 genotype and alelle proportion was in Hardy-Weinberg equilibrium in both study groups (Table 2). We observed a tendency for FGFR2 rs2981582 AA to be protective factor against oral cancer compared to GG genotype in codominant and recessive models, however, the results did not quite reach statistically significant level (p=0.334, Table 3).

FGFR2 rs2981582 genotype analysis by gender showed that rs2981582 AA genotype was expressed only in male patients but not in female with oral cancer diagnosis. Additionally, rs2981582 AA genotype was less frequent in males with oral cancer than in males from the control group; however, these differences were not statistically significant (Table 4).

Binomial logistic regression analysis did not reveal any associations between genotype and male or female gender in patients with oral cancer, and control group (Table 5).

Analysis of FGFR2 rs2981582 genotype distribution in different stages of cancer demonstrated that frequency of FGFR2 rs2981582 GG genotype was higher in patients with 3 or 4 stage oral cancer than in patients with stage 2 cancer, when AA genotype was more commonly detected in patients with stage 2 cancer (Table 6). Any associations between rs2981582 genotype and stages of cancer were found.

Further analysis showed a tendency of FGFR2 rs2981582 AA genotype to be most frequently determined in moderately differentiated (G2) tumors (Table 7). On the other hand, any statistically significant differences were found.

DISCUSSION

FGFs and their receptors (FGFRs) are a family of ligands and receptors that regulate tumor development, growth, differentiation, migration and angiogenesis (20). The FGF family has been described as having an impact on pituitary tumour activeness, aggressiveness and invasiveness (21-23). A total of 23 FGF ligands have been identified, so far They signal through four transmembrane tyrosine kinase receptors encoded by independent genes that each generates multiple isoforms. Each prototypic FGFR contains three Ig-like extracellular domains, a single transmembrane domain, a split tyrosine kinase cytoplasmic domain, and a COOH-terminal tail that typically contains tyrosines that are phosphorylated upon ligand binding and recruit intracellular signaling proteins. While some FGFs can signal through multiple receptors, the majority have a specific affinity for selected receptor isoforms (24).

The prognostic value of FGFR has been investigated in various types and localization of cancer. Our results showed that there were no associations between FGFR2 rs2981582 and oral cancer. However, further study to indentify the possible effect of FGFR on oral cancer with a larger sample size is required. Findings of other studies report about FGFR1 gene amplification and FGFR4 Gly388Arg polymorphism in lung SCC (25-31) and breast cancer (32-34). Studies on FGFR3 mutations in bladder cancer exclusively are in agreement with each other; nearly all these studies found FGFR2 rs2981582 correlations with better progression-free and disease-specific survival (35). Ipenbur et al. performed the initial search yielded 1568 publications of which 12 fulfilled the inclusion criteria. Four studies reported FGFR1 gene amplification (9.3-17.4%) and FGFR1 protein overexpression (11.8%) in head and neck squamous cell carcinoma (HNSCC). FGFR1 protein expression by cancer-associated fibroblasts correlated with poor survival outcome in one study (p<0.01) (36).

Eight studies reported high rates of FGFR4 Gly388Arg polymorphisms (32.5-54.2%) and FGFR4 protein overexpression , with varying correlations with survival (37-44). So far, no studies assessed the prognostic role of FGFR2, FGFR3, or FGFR5 in HNSCC. Thus, evidence was found for prognostic value only of FGFR1 expression in cancer-associated fibroblasts in HNSCC, so far. Prognostic evidence on the other FGFR family members in HNSCC is limited and conflicting. This emphasizes the need for future well-conducted prognostic studies (36). Other researchers state that FGFR-R388 is found in up to 50% of the population, and it has an impact on treatment of advanced or resistant breast cancer, colorectal cancer, prostate cancer, sarcomas, and head and neck cancer (45-48).

The FGFR genes are frequently aberrant in HNSCC; FGFR1 is amplified in 10% of HPV-negative HNSCC and FGFR3 is in 11% of HPV-positive HNSCC (49). Thus, HNSCC patients with FGFR-aberrated tumors may benefit from FGFR-inhibitor therapies as these tumors may be sensitive to treatment. Moreover, targeting FGFR family members has been shown enhanced sensitivity of cancer cells to radiotherapy and chemotherapy treatment. Radiotherapy resistant cancer cells upregulate FGFR3 protein when chemoradiotherapy resistant cancer cells – FGFR4 protein. Targeting FGFR3 in resistant HNSCC cells restored sensitivity to radiotherapy and targeting FGFR4-sensitivity to chemo-radio therapy (50-52).

FGFRs are upcoming promising therapeutic targets and possible prognostic biomarkers in multiple types of cancer, including HNSCC (53). The FGFR family comprises five (FGFR1-5) cell membrane-

bound tyrosine kinase receptors linked to multiple intracellular downstream signaling pathways. FGFRs regulate tissue homeostasis in normal human tissues (54, 55). However, the molecular mechanisms through which FGFR2 amplification promotes lymph node metastasis remain unclear (56, 57).

CONCLUSION

Results of present study showed no association between FGFR2 gene polymorphisms rs2981582 and oral cancer. However, a further study with a larger sample sizes is advisable.

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