SPANDIDOS PUBLICATIONS

FGFR4 polymorphism, *TP53* mutation, and their combinations are prognostic factors for oral squamous cell carcinoma

JUN-ICHI TANUMA[1], TOSHIYUKI IZUMO[2], MASATO HIRANO[1], YOSHITAKA OYAZATO[1], FUMIYA HORI[1], ERI UMEMURA[1], HAYASE SHISA[2], HIROSHI HIAI[3] and MOTOO KITANO[4]

[1]Department of Oral Pathology, Field of Oncology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8544; [2]Department of Pathology, Saitama Cancer Center Research Institute, Ina, Saitama 362-0800; [3]Shiga Medical Center, Moriyama, Shiga 520-8501; [4]Department of Medical Research and Clinical Pathology, Nakatsu Municipal Hospital, Nakatsu 871-8511, Japan

Received July 20, 2009; Accepted October 5, 2009

DOI: 10.3892/or_00000692

Abstract. The genotype of the fibroblast growth factor receptor 4 (*FGFR4*) gene and *TP53* mutation have been reported as prognostic factors for cancers of the head and neck, bladder, breast and colon. To determine whether they are applicable for oral squamous cell carcinoma (OSCC), we investigated these two genes in OSCC samples from 150 patients who had undergone radical surgery and in 100 cancer-free individuals. In OSCC, the *FGFR4* Gly388Arg polymorphism and the presence or absence of mutation in *TP53* did not show a significant association with the clinicopathological features of the tumors at surgery. However, the *FGFR4* Arg388 allele, as well as mutations in *TP53*, was found to be closely associated with poor prognosis. Moreover, these two parameters synergistically affected the survival of OSCC patients. During 60 months of observation after radical surgery, a majority of patients with homozygous Arg388 *FGFR4* plus mutated *TP53* died of cancer, whereas >90% patients carrying homozygous Gly388 *FGFR4* plus wild-type *TP53* survived. Therefore, the *FGFR4* Gly388Arg polymorphism and *TP53* mutations, as well as their combinations, are excellent predictors of the prognosis for OSCC patients.

Introduction

More than 500,000 people are diagnosed every year worldwide with head and neck cancer, including the cancers of the larynx, nasal passages/nose, oral cavity and pharynx (1). Oral squamous cell carcinoma (OSCC) is prone to local recurrence and distant metastasis. These properties are major hazards to establish an effective therapeutic strategy. To explore reliable molecular parameters to predict OSCC prognosis, we selected two genes. The mutations in the *TP53* gene, which occur in >50% of OSCC, especially in advanced tumors (2,3), and the polymorphism in the *FGFR4* gene, which is a member of the family of fibroblast growth factor receptors (FGFRs). FGFRs are shown to interact with >20 known ligands and to display multiple biological activities, including mitogenic and angiogenic activities. Dysregulation of this pathway has been demonstrated in several tumor types (4-6). In the human *FGFR4* gene, a single nucleotide polymorphism in exon 9 results in an amino acid change (Gly388Arg) in the transmembrane domain. The *FGFR4* Arg388 allele has been reported to be a promising prognostic factor in some human cancers, including cancers of the breast (6,7), colon (7), lung (8), prostate (9) and head and neck (10,11) and in soft tissue sarcomas (12). However, its significance is still controversial (13,14).

To evaluate the prognostic significance of *FGFR4*, *Tp53* and their combinations in OSCC, we studied the genotypes of these genes in Japanese OSCC patients and control individuals, the clinicopathological parameters and the prognosis. Possible correlations among these parameters were analyzed.

Materials and methods

Patients. The study included patients suffering from OSCC who underwent surgery at Saitama Cancer Center Hospital and control patients without OSCC enrolled at the same Institution and the Kagoshima University Hospital. Clinicopathological data for all OSCC patients are summarized in Table I.

The group of OSCC consisted of 150 individuals (80 males and 70 females) aged 35 to 80 years (median 55.5) with primary cancers of the tongue (77), gingival (35), buccal mucosa (28) and floor of the mouth (10). The age of the 100 controls (55 males and 45 females) ranged from 31 to 85 years (median 60.2).

Genomic DNAs were extracted from the fresh tumor tissues and the peripheral lymphocytes in blood samples. For the control, genomic DNAs were obtained from the peripheral

Correspondence to: Dr Jun-ichi Tanuma, Department of Oral Pathology, Field of Oncology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8544, Japan
E-mail: tanuma@dent.kagoshima-u.ac.jp

Key words: fibroblast growth factor receptor 4, polymorphism, *TP53*, oral squamous cell carcinoma

Table I. Correlation among the *FGFR4* polymorphism, *TP53* mutation and clinicopathological features of OSCC patients.

Characteristics	No. of patients (%)	*FGFR4* allelotype			*TP53* mutation	
		Gly/Gly	*Gly/Arg*	*Arg/Arg*	Yes[a]	No
All patients (%)	150 (100)	69 (46.0)	53 (35.3)	28 (18.7)	55 (36.7)	95 (63.3)
Age (yrs)						
<55	71 (47.3)	33	25	13	25	46
>55	79 (52.7)	36	28	15	30	49
P-value			0.991		0.725	
Gender						
Male	80 (53.3)	36	24	20	33	47
Female	70 (46.7)	33	31	8	22	48
P-value			0.078		0.213	
Smoking status						
Current	50 (33.3)	20	16	14	15	35
Former	75 (50.0)	35	30	10	30	45
Never	25 (16.7)	14	7	4	10	15
P-value			0.238		0.487	
Tumor site						
Tongue	77 (51.3)	32	30	15	34	43
Another region	73 (48.7)	37	23	13	21	52
P-value			0.516		0.061	
T classification						
T1 + T2	49 (32.7)	29	13	7	20	29
T3 + T4	101 (67.3)	40	40	21	35	66
P-value			0.078		0.462	
Mode of invasion						
Grade 1 + 2	45 (30.0)	25	14	6	21	24
Grade 3 + 4	105 (70.0)	44	39	22	34	71
P-value			0.275		0.101	
Vascular invasion						
Positive	59 (39.3)	25	22	12	22	37
Negative	91 (60.7)	44	31	16	33	58
P-value			0.767		0.898	
Lymph node involvement						
Positive	51 (34.0)	25	16	10	23	28
Negative	99 (66.0)	44	37	18	32	67
P-value			0.766		0.124	

[a]Yes means the number of cases carrying any of the TP53 mutations.

lymphocytes in blood samples or stored specimens fixed in formalin and embedded in paraffin using the QIAamp Tissue Extraction Kit (Qiagen, Chatsworth, CA, USA).

The present study was performed under approval of the Ethics Committees of both the Saitama Cancer Center (No. 49) and the Kagoshima University Dental School (No. 1).

Genotyping. Samples were automatically genotyped for the *FGFR4* Gly388Arg polymorphism (rs351855) employing the ABI Prism SNaPshot Multiplex kit according to the manufacturer's recommendations (ABI, Foster City, CA, USA). Briefly, the genomic DNA flanking the SNP was amplified with PCR with forward primer AGG TGT GGG TGC CTG GGA CT and reverse primer GGG AAC TCC CAT AGT GGG TCG. The PCR mix contained 25 ng of genomic DNA, 1 μl of 10X PCR Gold buffer, 200 μM dNTPs, 3 mM $MgCl_2$, 0.5 U of AmpliTaq Gold Polymerase (ABI) and 1 pM of each primer. PCR was carried out as follows: 10 min at 95˚C for 1 cycle

Hazard ratios of death for the *FGFR4* polymorphism and *TP53* mutation status in OSCC patients.

Genotype	No. of patients	No. of patients who died	Harzard ratio[a]	95% CI	P-value
FGFR4 at amino acid 388					
Gly/Gly	69	18	1		
Gly/Arg	53	25	1.15	0.58-2.49	0.048
Arg/Arg	28	17	1.43	1.15-3.01	0.025
TP53					
Wild-type	95	25	1		
Mutant	55	35	1.38	1.21-2.85	0.031
FGFR4: *TP53*					
Gly/Gly: Wild-type	38	3	1		
Gly/Gly: Mutant	31	15	1.29	0.68-2.93	0.067
Gly/Arg: Wild-type	40	15	1.31	0.99-2.56	0.065
Gly/Arg: Mutant	13	10	1.39	1.11-2.64	0.046
Arg/Arg: Wild-type	17	7	1.41	1.21-2.96	0.028
Arg/Arg: Mutant	11	10	1.69	0.87-2.87	0.001

[a]Hazard ratios were adjusted for age, gender, lymph node involvement, tumor stage and grade.

and 35 cycles at 95˚C for 30 sec, 58˚C for 40 sec and 72˚C for 1 min, followed by 1 cycle at 72˚C for 7 min. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche) and exonuclease I (USB Corporation) at 37˚C for 60 min and 72˚C for 15 min to purify the amplified products. One μl of the purified amplification products was added to SNaPshot Multiplex Ready reaction mixture containing 0.15 pmol of genotyping primer CTG CCC TCG ATA CAG CC for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96˚C for 10 sec, 50˚C for 5 sec and 60˚C for 30 sec. The reaction products were treated with 1 unit of SAP at 37˚C for 1 h and 72˚C for 15 min to remove excess fluorescent dye terminators. One μl of the final reaction samples containing the extension products was added to 9 μl of Hi-Di formamide (ABI). The mixture was incubated at 95˚C for 5 min, followed by 5 min on ice and then analyzed by electrophoresis in ABI 310. Results were analyzed using GeneScan analysis software (ABI).

PCR-SSCP analysis and DNA sequencing. Single-stranded conformation polymorphism (SSCP) analysis was used to analyze tumor samples for mutations within exons 4-10 of the *TP53* gene as described (15,16). Cases displaying an altered electrophoretic mobility were reamplified and analyzed by direct sequencing of both strands to confirm and characterize the nature of the mutation.

Statistical analysis. Molecular genetic analysis was performed without knowledge of the clinical data, which were disclosed after completion of the marker analysis. Logistic regression was used to compute odds ratios (ORs) and 95% confidence intervals (CIs). The power of the study design was calculated according to Dupont and Plummer (17). The Kaplan-Meier product limit method and the log-rank test were used to estimate survival functions (18,19). Hardy-Weinberg equilibrium was tested by the Chi-square method (20). Based on the extracted binary data, odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for the association between the *FGFR4* polymorphism and *TP53* mutations. Statistical procedures were carried out with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software (21).

Results

Genotype of FGFR4 and TP53 in OSCC patients. The allele types of *FGFR4* amino acid 388 in 150 OSCC patients were Arg/Arg (15.3%), Arg/Gly (38.7%) and Gly/Gly (46.0%), while those in 100 control individuals were Arg/Arg (10.0%), Arg/Gly (48.0%) and Gly/Gly (42.0%). The odds ratio for the OSCC patients vs. control for the Arg388 allele was 1.15 (95% CI, 0.5-2.1). Therefore, the risk for OSCC development did not show an association with the *FGFR4* genotype. In addition, the *FGFR4* Gly388Arg genotypes did not show any obvious correlation with the clinicopathological parameters at the time of initial treatment (Table I).

On the other hand, the mutations of the *TP53* gene were detected by SSCP in 55 of 150 OSCC samples (36.7%). There were eight types of mutations of the *TP53* gene in these samples: 9 cases in exon 5 (TGC/TAC transition at codon 135, leading to Cys/Phe substitution), 8 in exon 5 (CCG/CTG at codon 152, Pro/Leu substitution), 5 in exon 5 (CGC/CAC at codon 17, Arg/His substitution), 4 in exon 6 (TTG/TAG at codon 201, Leu/stop codon), 10 in exon 7 (CGC/AGC at codon 245, Gly/Ser substitution), 8 in exon 7 (CCG/CTG at codon 248, Arg/Gln substitution), 10 in exon 8 (CGT/TGT at codon 273, Arg/Cys substitution) and 1 in exon 9 (CAG /TAG at codon 317, Gly/stop codon). Although these mutations

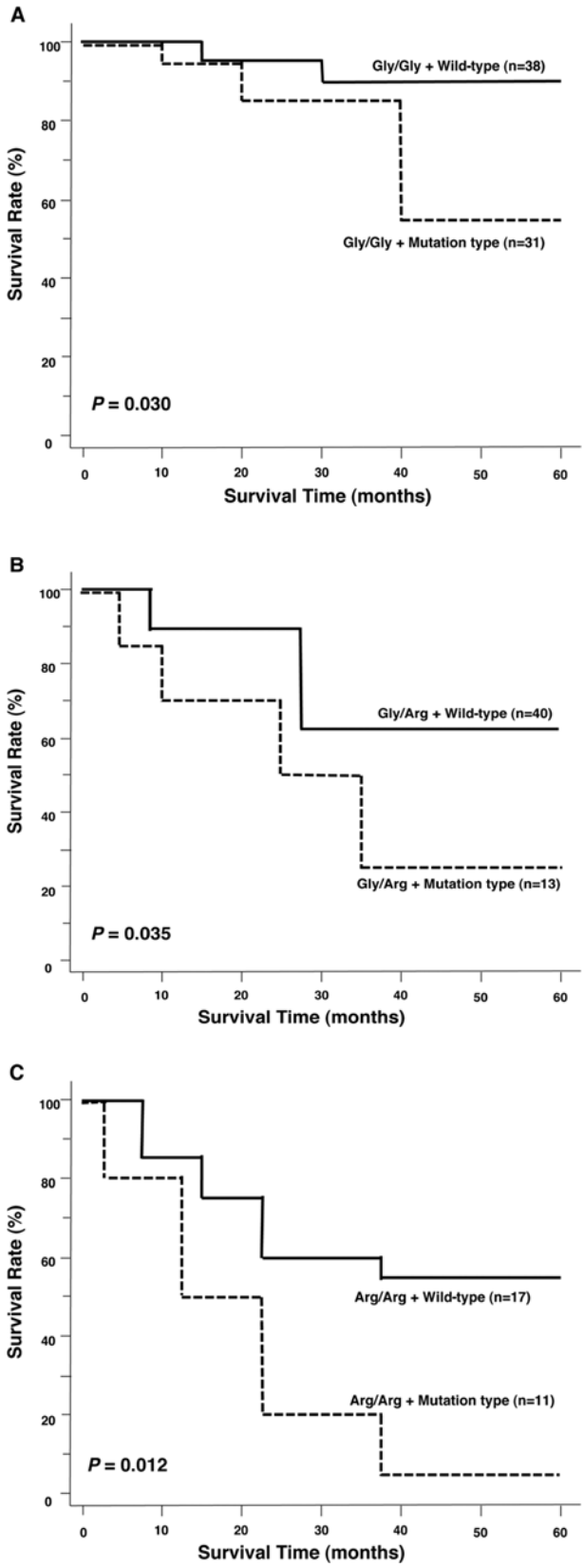

Figure 1. Kaplan-Meier survival curves in OSCC patients. The solid lines represent wild-type *TP53* and the broken lines, mutant *TP53*. (A) *FGFR4* allele at codon 388 Gly/Gly. Log-rank analysis indicates significant differences between the patients with wild-type *TP53* and those with mutant *TP53* mutations (P=0.030). (B) *FGFR4* allele, Gly/Arg. The difference between wild-type and mutant *TP53* was significant (P=0.035). (C) *FGFR4* allele, Arg/Arg. The difference between wild-type and mutant *TP53* was significant (P=0.012). The difference between the patients having the *FGFR4* allele Gly/Gly and wild-type *TP53* and those having the *FGFR4* allele Arg/Arg and mutant *TP53* was highly significant (P=0.001).

caused amino acid substitutions or the generation of a stop codon, none of them was specifically associated with the clinicopathological parameters at the initial treatment.

Prognosis of OSCC patients by genotypes. Of the 150 patients who underwent a radical operation, 60 (40.0%) died of OSCC recurrence within the 60 month follow-up period. The Cox proportional hazard analysis of survival, adjusted for gender, smoking and age at diagnosis (in decennia) showed that the patients carrying *FGFR4* allele Gly/Arg or Arg/Arg at amino acid 388 exhibited a much higher hazard ratio (HR) than the patients with *FGFR4* allele Gly/Gly (Table II). The median survival period for Arg allele carriers was 34 months, whereas that for Gly/Gly OSCC patients was 78 months. Table II also shows that the patients with OSCC carrying the *TP53* mutation had higher HR (1.38) than those with wild-type *TP53*. There were minor differences in the survival rate among the types of *TP53* mutation, but the number of each type was insufficient for statistical analysis. In this study, therefore, all the *TP53* mutations were handled as a single group.

Combined prognostic effects of FGFR4 and TP53. The genotype combinations of both markers showed stronger association with HR (Table II) and survival rates (Fig. 1) than the genotype of each marker. Fig. 1 shows the survival curves of patients with *FGFR4* of (A) Gly/Gly allele, (B) Gly/Arg, or (C) Arg/Arg allele. In all three groups, the patients having a *TP53* mutation showed a significantly lower rate of survival than those with wild-type *TP53* by Kaplan-Meier analysis. The most striking difference was seen between the patients with *FGFR4* Arg/Arg plus *TP53* mutations (median survival time = 20.5 months) and the patient group with *FGFR4* Gly/Gly plus wild-type *TP53* (median survival time = 34.7 months).

Discussion

The present study demonstrated that two genetic parameters, the *FGFR4* Gly388Arg polymorphism and mutations in *TP53* and their combinations are significant predictors for the fate of OSCC patients after radical surgery.

The mechanism by which *FGFR4* affects cancer progression is poorly understood. *FGFR4* is implicated in signaling cascades with regard to cell-matrix adhesion and angiogenesis. These functions include the up-regulation of a urokinase-type plasminogen activator (uPA), a proteolytic enzyme, required for cell migration (22) and the association with N-CAM in cell adhesion to the extracellular matrix (23). Not surprisingly, abnormal expression of *FGFR4* has been suggested as a potential mechanism in the progression of cancers in several organs (24,25). On the other hand, its single nucleotide polymorphism at codon 388 has been reported to be relevant to cancer prognosis. Bange *et al* (6) reported that the progression, tumor cell motility, metastasis and reduced disease-free survival of breast cancer patients are associated with the *FGFR4* Arg388 allele, although this finding remains controversial (7,14,23). In prostate cancer (9), lung cancer (8), colorectal cancer (7), head and neck cancers (10,11) and soft tissue sarcomas (12), the presence of the *FGFR4* Arg388 allele has been shown to indicate a poor

. The present data confirmed that it is a promising ant for the prognosis of an OSCC patient. Although there was no significant correlation between the genotype of *FGFR4* and the clinicopathological parameters of OSCC at the first treatment, the presence of the *FGFR4* Arg388 allele reduced the patient survival rate in a dose-dependent manner.

The *FGFR4* Gly388Arg polymorphism results in an amino acid change in the transmembrane domain, a highly conserved region for receptor tyrosine kinase. Analogous missense mutations in the transmembrane domain in the *FGFR3* gene, resulting from a Gly to Arg substitution at codon 380, were proposed to result in constitutive activation of *FGFR3* signaling (26). In general, activated *FGFR3* mutations are associated with favorable disease characteristics, such as low stage and grade, low recurrence rate and a lower mortality rate (27,28). Therefore, the *FGFR4* Arg388 allele could augment the aggressive behavior of cancer cells in OSCC, although it remains obscure if *FGFR4* Arg388 is more activated than its Gly388 form.

It has been previously noted that the *FGFR4* Arg388 allele increases *FGFR4* expression (6). Streit *et al* (10) reported that *FGFR4* expression *per se* was not associated with a worse prognosis in head and neck SCC. However, among the patients with high *FGFR4* expression, the overall survival was significantly more reduced in those with the Arg388 allele than in those with the Gly allele. Although we did not evaluate the expression of *FGFR4* in the present series, further study should be conducted to determine whether the role of the *FGFR4* polymorphism in OSCC progression is the result of an activated function of the receptor, increased expression, or their combination.

We also showed the *TP53* mutations increased the hazard ratio and reduced the survival rate of OSCC patients. The importance of the *TP53* tumor suppressor gene in the process of carcinogenesis is well established. *TP53* controls several key pathways that protect cells from malignant transformation. Approximately 40-50% of OSCC has alternations in *TP53* (16), which regulates the cellular stress response pathway that has been shown to be critical for the maintenance of genomic integrity. In particular, a high incidence of the *TP53* mutation has been demonstrated in tobacco-related cancers (29-31).

The most striking finding of this study is that these two predictors act jointly to reduce the survival rate of OSCC patients. The observation supports the hypothesis in which the *FGFR4* and *TP53* gene mutations may represent alternative genetic pathways in the progression of OSCC. The joint action of the *FGFR4* genotype and *TP53* has also been reported in bladder cancer patients (32). In this case, neither the *FGFR4* polymorphism nor the *TP53* mutation status was an independent predictor of prognosis, but a combination of *FGFR4* Gly388 plus *TP53* mutation is associated with a poor probability of survival. The apparently reverse function of *FGFR4* alleles on cancer progression suggests that their biological action may be organ-specific.

In conclusion, the *FGFR4* polymorphism and *TP53* mutation status are useful prognostic factors for OSCC. Their combination, in particular, is a highly reliable and useful predictor in clinical situations.

Acknowledgements

This study was supported by a Grant-in-Aid from the Japanese Ministry of Education, Science, Sports and Culture of Japan (No. 18659558, 21592393).

References

1. American Cancer Society Cancer facts and figures, 2006 Report. Available from: http://www.cancer.org/
2. Zhang Y, Hiraishi Y, Wang H, *et al*: Constitutive activating mutation of the FGFR3b in oral squamous cell carcinomas. Int J Cancer 17: 166-168, 2005.
3. Aubertin J, Tourpin S, Janot F, Ahomadegbe JC and Radvanyi F: Analysis of fibroblast growth factor receptor 3 G697C mutation in oral squamous cell carcinomas. Int J Cancer 120: 2058-2059, 2007.
4. Powers CJ, McLeskey SW and Wellstein A: Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 7: 165-197, 2000.
5. Beau-Faller M, Gaub MP, Schneider A, *et al*: Allelic imbalance at loci containing FGFR, FGF, c-Met and HGF candidate genes in non-small cell lung cancer sub-types, implication for progression. Eur J Cancer 39: 2538-2547, 2003.
6. Bange J, Prechtl D, Cheburkin Y, *et al*: Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. Cancer Res 62: 840-847, 2002.
7. Spinola M, Leoni VP, Tanuma J, *et al*: FGFR4 Gly388Arg polymorphism and prognosis of breast and colorectal cancer. Oncol Rep 14: 415-419, 2005.
8. Spinola M, Leoni V, Pignatiello C, *et al*: Functional *FGFR4* Gly388Arg polymorphism predicts prognosis in lung adenocarcinoma patients. J Clin Oncol 23: 7307-7311, 2005.
9. Wang J, Stockton DW and Ittmann M: The fibroblast growth factor receptor-4 Arg388 allele is associated with prostate cancer initiation and progression. Clin Cancer Res 10: 6169-6178, 2004.
10. Streit S, Bange J, Fichtner A, Ihrler S, Issing W and Ullrich A: Involvement of the FGFR4 Arg388 allele in head and neck squamous cell carcinoma. Int J Cancer 111: 213-217, 2004.
11. da Costa Andrade VC, Parise O Jr, Hors CP, de Melo Martins PC, Silva AP and Garicochea B: The fibroblast growth factor receptor 4 (FGFR4) Arg388 allele correlates with survival in head and neck squamous cell carcinoma. Exp Mol Pathol 82: 53-57, 2007.
12. Morimoto Y, Ozaki T, Ouchida M, *et al*: Single nucleotide polymorphism in fibroblast growth factor receptor 4 at codon 388 is associated with prognosis in high-grade soft tissue sarcoma. Cancer 98: 2245-2250, 2003.
13. Becker N, Nieters A and Chang-Claude J: The fibroblast growth factor receptor gene Arg388 allele is not associated with early lymph node metastasis of breast cancer. Cancer Epidemiol Biomarkers Prev 12: 582-583, 2003.
14. Jézéquel P, Campion L, Joalland MP, *et al*: G388R mutation of the FGFR4 gene is not relevant to breast cancer prognosis. Br J Cancer 90: 189-193, 2004.
15. Tanuma J, Hiai H, Shisa H, *et al*: Carcinogenesis modifier loci in rat tongue are subject to frequent loss of heterozygosity. Int J Cancer 102: 638-642, 2002.
16. Hsieh LL, Wang PF, Chen IH, *et al*: Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. Carcinogenesis 22: 1497-1503, 2001.
17. Dupont WD and Plummer WD Jr: Power and sample size calculations: a review and computer program. Control Clin Trials 11: 116-128, 1990.
18. Kaplan EL and Meier P: Non-parametric estimation from incomplete observations. J Am Stat Assoc 3: 457-481, 1958.
19. Peto R and Peto J: Asymptotically efficient rank invariant test procedures. JR Stat Soc [A] 135: 185-206, 1972.
20. Weir BS: Genetic Data Analysis 2. Methods for Discrete Population Genetic Data. Sinauer Associates Inc, Sunderland, 1996.
21. Lohmueller KE, Pearce CL, Pike M, Lander ES and Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 33: 177-182, 2003.
22. Boilly B, Vercoutter-Edouart AS, Hondermarck H, Nurcombe V and Bourhis X: FGF signals for cell proliferation and migration through different pathways. Cytokine Growth Factor Rev 11: 295-302, 2000.

23. Cavallaro U and Christofori G: Multitasking in tumor progression: Signaling functions of cell adhesion molecules. Ann NY Acad Sci 1014: 58-66, 2004.
24. Leung HY, Gullick WJ and Lemoine NR: Expression and functional activity of fibroblast growth factors and their receptors in human pancreatic cancer. Int J Cancer 59: 667-675, 1994.
25. Jaakkola S, Salmikangas P, Nylund S, *et al*: Amplification of the FGFR4 gene in human breast and gynaecological cancers. Int J Cancer 54: 378-382, 1983.
26. Webster MK and Donoghue DJ: Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. EMBO J 15: 520-527, 1996.
27. Sibley K, Cuthbert-Heavens D and Knowles MA: Loss of heterozygosity at 4p16.3 and mutation of *FGFR3* in transitional cell carcinoma. Oncogene 20: 4416-4418, 2001.
28. van Rhijn BW, Lurkin I, Radvanyi F, Kirkels WJ, van der Kwast TH and Zwarthoff EC: The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. Cancer Res 61: 1265-1268, 2001.
29. Boyle JO, Hakim J, Koch W, *et al*: The incidence of *p53* mutations increases with progression of head and neck cancer. Cancer Res 53: 4477-4480,1993.
30. Ostwald C, Gogacz P, Hillmann T, *et al*: *p53* mutational spectra are different between squamous cell carcinomas of the lip and the oral cavity. Int J Cancer 88: 82-86, 2000.
31. Saranath D, Tandle AT, Teni TR, *et al*: p53 inactivation in chewing tobacco-induced oral cancers and leukoplakias from India. Oral Oncol 35: 242-250, 1999.
32. Yang YC, Lu ML, Rao JY, *et al*: Joint association of polymorphism of the *FGFR4* gene and mutation *TP53* gene with bladder cancer prognosis. Br J Cancer 95: 1455-1458, 2006.