

INVOLVEMENT OF THE FGFR4 Arg388 ALLELE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Sylvia STREIT¹, Johannes BANGE², Alexander FICHTNER, Stephan IHRLER³, Wolfgang ISSING⁴ and Axel ULLRICH^{1*}

¹Department of Molecular Biology, Max Planck Institute for Biochemistry, Martinsried, Germany

²U3 Pharma AG, Martinsried, Germany

³Department of Pathology, University of Munich, Munich, Germany

⁴Department of ENT, Freeman Hospital, Newcastle Upon Tyne, United Kingdom

⁵Department of Otorhinolaryngology, Klinikum Grosshadern, Munich, Germany

Fibroblast growth factor receptors (FGFRs) have been implicated in various forms of human hyperproliferative disorders such as cancers of the cervix and bladder. We investigated the expression pattern of FGFR4 and the clinical significance of the recently identified Gly/Arg polymorphism (388) in head and neck squamous cell carcinomas (HNSCCs) of the oral cavity and the oropharynx. Sections from 104 paraffin-embedded tumors were analyzed by a restriction fragment length polymorphism-based method to determine the FGFR4 genotypes. Protein expression was investigated immunohistochemically and graded into a low, intermediate, or high degree of staining. FGFR4 expression was scored as high in 17, as intermediate in 59 and as low in 28 cases. The FGFR4 Arg388 allele was found in 59 tumors, 46 of them having heterozygous and 13 homozygous genotypes. High expression of the FGFR4 Arg388 allele was significantly associated with reduced overall survival ($p = 0.032$) and with an advanced tumor stage ($p = 0.023$), whereas expression of the FGFR4 Gly388 had no impact on disease progression. Our findings indicate that high expression of FGFR4 in connection with the Arg388 allele is associated with poor clinical outcome and support the significance of FGFR4 as a diagnostic marker and a target for therapeutic intervention in human HNSCC.

© 2004 Wiley-Liss, Inc.

Key words: fibroblast growth factor receptor 4; head and neck squamous cell carcinoma; FGFR4 Arg388 allele

After decades of rigorous investigations of chemotherapeutic cancer therapies, many cancers, including those of the head and neck, remain beyond our clinical ability to control them. From 3% to 5% of all patients initially cured of early-stage head and neck cancer will develop second primary tumors or local recurrences.¹ This phenomenon has been explained by the concept of field cancerization, which argues that certain risk factors such as alcohol and tobacco change the lining of the upper aerodigestive tract into a so-called condemned mucosa. In such a scenario, alcohol may represent the key risk factor for neoplastic transformation in the oral cavity, the oro- and hypopharynx.^{2–4} An increasing amount of evidence suggests that alcohol intake and smoking play a synergistic role in the neoplastic progress.

One of the most important achievements of molecular oncology has been the demonstration that cancer represents a genetic disease that begins with genetic damages in the genome of one cell in the form of point mutations, DNA rearrangement and gene amplification leading to the distortion of the expression and biochemical function of respective gene products. Growth factor receptors of the class I subfamily, which include the epidermal growth factor receptor (EGFR/HER1) and the related proteins HER2, HER3 and HER4, have shown that amplification or overexpression of those receptors, especially well documented for HER2 in patients with breast and ovarian cancers, plays an important role in carcinogenesis and tumor progression.^{5–8} Multivariate survival analysis showed that HER2 amplification or overexpression is more predictive of clinical outcome than all other known prognosticators with the exception of positive lymph nodes.^{9,10} These findings have validated the HER2 receptor as a target for therapeutic intervention. Moreover, recent clinical investigations have con-

firmed the benefit of antagonistic HER2 antibody therapy in breast cancer using herceptin.¹¹ Minute genetic changes such as point mutations have been found to cause constitutive activation of the HER2/neu kinase activity and to induce neoplastic disorders.¹² Similarly, the 2 amino acid substitutions Gly380Arg and Ala391Glu in the transmembrane domain of fibroblast growth factor receptor 3 (FGFR3) have been shown to be responsible for 2 forms of human dwarfism, namely, achondroplasia and morbus crouzon.^{13–15} Other mutations in the FGFR3 gene have been associated with bladder and cervix cancer.¹⁶ The FGFR signaling system is composed of 4 receptor tyrosine kinases (RTKs) and more than 20 known ligands and has been implicated in the regulation of a multitude of both physiologic and pathophysiologic processes, including migration, wound healing, angiogenesis and cancer.¹⁷ More recently, we have identified a single nucleotide polymorphism (SNP) in codon 388 of the FGFR4 gene, which plays a pivotal role in the progression of breast cancer. This SNP is present at a significantly higher rate in breast cancer patients with early relapse and represents therefore a gene alteration that critically impacts the survival time of patients.¹⁸

In order to determine whether the SNP in FGFR4 codon 388 plays a role in tumor progression of other human cancers, we analyzed FGFR4 expression levels and the presence of the Arg388 allele in the FGFR4 gene in 104 squamous cell carcinomas of the oral cavity and the oropharynx and analyzed its correlation with clinical parameters.

MATERIAL AND METHODS

Tissue specimens

Surgical specimens from 104 cases of head and neck squamous cell carcinoma (HNSCC) were retrieved from the Institute of Pathology of the Ludwigs Maximilians University of Munich (from years 1984–1998). The tissue sample collection comprises 84 men and 20 women ranging in age from 38 to 81 years. Patients had not received irradiation or anticancer chemotherapy before surgery. All patients were staged according to the UICC pTNM classification. Median follow-up in patients at the time of analysis was 39 month. Of the 104 patients, 40 died in the follow-up period. Each specimens was fixed in 4% buffered formaldehyde, embedded in paraffin wax and cut into 4 μ m thick sections for further analysis.

*Correspondence to: Department of Molecular Biology, Max Planck Institute for Biochemistry, Am Klopferspitz 18A, D-82152 Martinsried, Germany. Fax: +49-89-8578-2454. E-mail: ullrich@biochem.mpg.de

Received 4 December 2003; Revised 14 January 2004; Accepted 27 January 2004

DOI 10.1002/ijc.20204
Published online 12 April 2004 in Wiley InterScience (www.interscience.wiley.com).

Immunohistochemistry

Immunostaining was performed using a sensitive 3-layer avidin-biotin complex (ABC) method with the rabbit IgG Vectastain Elite ABC (peroxidase) kit as outlined by the manufacturer (Vector Laboratories). Paraffin sections were dewaxed in xylene and hydrated through graded alcohols to water and subsequently placed in PBS. The sections were incubated for 10 min with 1% hydrogen peroxide to inhibit endogenous peroxidase. After rinsing with PBS, sections were pretreated with citrate buffer, pH 6, for 12 min in a microwave oven at 700 W. Before incubation with normal goat serum, sections were immersed in PBS. Immunohistochemical staining was performed with a polyclonal antibody (clone 16; Santa Cruz, Santa Cruz, CA) to FGFR4. Primary antiserum was diluted 1:1,000 in PBS/1% BSA. The specificity of the rabbit polyclonal antibody has been determined by preincubation with a control peptide (dilution 1:100; Santa Cruz). After rinsing in PBS, sections were incubated with a biotinylated goat antirabbit antibody for 1 hr. Sections were rinsed with PBS and incubated for 30 min with the ABC solution. After further washes in PBS, the reaction product was visualized using diaminobenzidine (Sigma, St. Louis, MO) as chromogen. Sections were counterstained with Mayer's hematoxylin and mounted.

PCR-based genotyping

Genomic DNA was prepared from paraffin-embedded tumor tissue by standard protocols.¹⁹ Each paraffin section was extracted twice with xylene to remove the paraffin. This organic extraction was followed by 2 washes with 100% ethanol to remove the solvent. The ethanol was removed by rinsing the samples with acetone. Extracted and dried samples were then digested with proteinase K.

For screening individuals for the FGFR4 Arg388, the following primers were designed: 5'-GACCGCAGCAGCGCCGAGGC-CAG-3' and 5'-AGAGGGAAGAGGGAGAGCTTCTG-3'. Primers (2 μ M) and genomic DNA were combined in a 25 μ l total reaction volume using Ready-to-Go PCR beads (Pharmacia, Uppsala, Sweden). After denaturing for 3 min at 95°C, the reaction mixture was subjected to 35 cycles of 45 sec at 95°C and 45 sec at 72°C followed by 1 cycle at 72°C for 5 min. The 168 bp fragment was digested overnight with BstNI (New England Biolabs, Beverly, MA) according to the manufacturer's instruction. Restriction fragments were resolved on a 12% nondenaturing polyacrylamide

gel and DNA was visualized with ethidium bromide. The Arg388 allele was characterized by 2 distinctive fragments of 80 and 29 bp, whereas a single distinctive band of 109 bp was observed for the Gly388 allele.

Statistical analysis

Statistical evaluations were performed using the MedCalc package (MedCalc Software, Mariakerke, Belgium) and WinStat. The frequencies of the genotypes among different subgroups were calculated by *p*-values to assess the association between FGFR4 Arg388 and malignancy in the volunteers. Survival curves for overall survival were plotted according to Kaplan-Meier and compared using log-rank statistics. All test were performed at a significance level of $\alpha = 0.05$.

RESULTS

FGFR4 protein expression in tumors of oral cavity

FGFR4 is widely expressed in different nonneoplastic epithelial and mesenchymal tissues.²⁰ In agreement with this, we found the most intense immunohistochemical FGFR4 staining in the arterial smooth muscle cells and in skeletal muscle (Fig. 1). A low to moderate degree of staining was seen in fibroblasts and the ductal epithelium of small salivary glands. Due to the constant high staining intensity and their overall availability, medium-sized vessels and skeletal muscle were used as internal control for the grading of FGFR4 expression. In the invasive tumor areas, the cytoplasmic expression for the staining against FGFR4 was semi-quantitatively evaluated in a 3-graded system: low, intermediate and high expression (Fig. 2a and b). In control experiments, staining was abolished when the primary FGFR4 antibody was preincubated with the corresponding blocking peptide (Fig. 2c and d). No tumor was observed with absolutely no FGFR4 staining. The results of the FGFR4 protein expression pattern in the invasive tumor areas are summarized in Table I.

FGFR4 genotype distribution in patients with HNSCC

Recently, a strong correlation between the presence of the FGFR4 Arg388 allele and clinical and pathologic parameters for poor prognosis in breast and colon cancer patients was observed.¹⁸ Since the analysis of FGFR4 expression showed that this receptor is widely expressed in tumor specimens from the oral cavity and

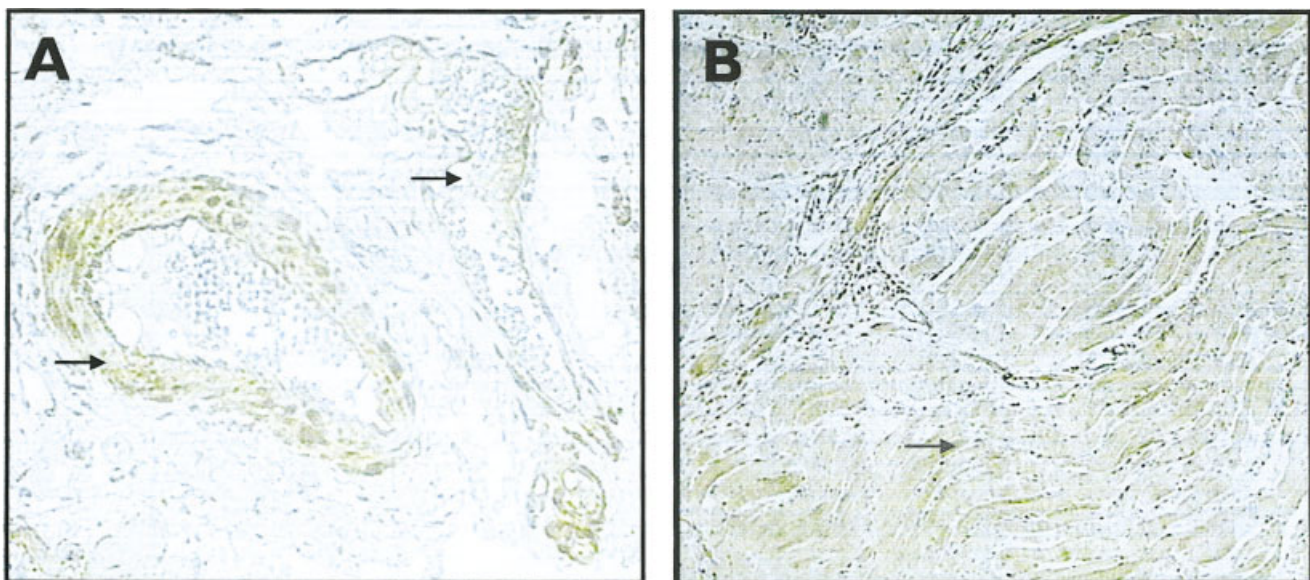


FIGURE 1 – Immunohistochemic staining of FGFR4. (a) FGFR4 immunoreactivity in arterioles and veins and (b) in skeletal muscles (original magnification, $\times 20$). Counterstaining with hematoxylin and eosin.

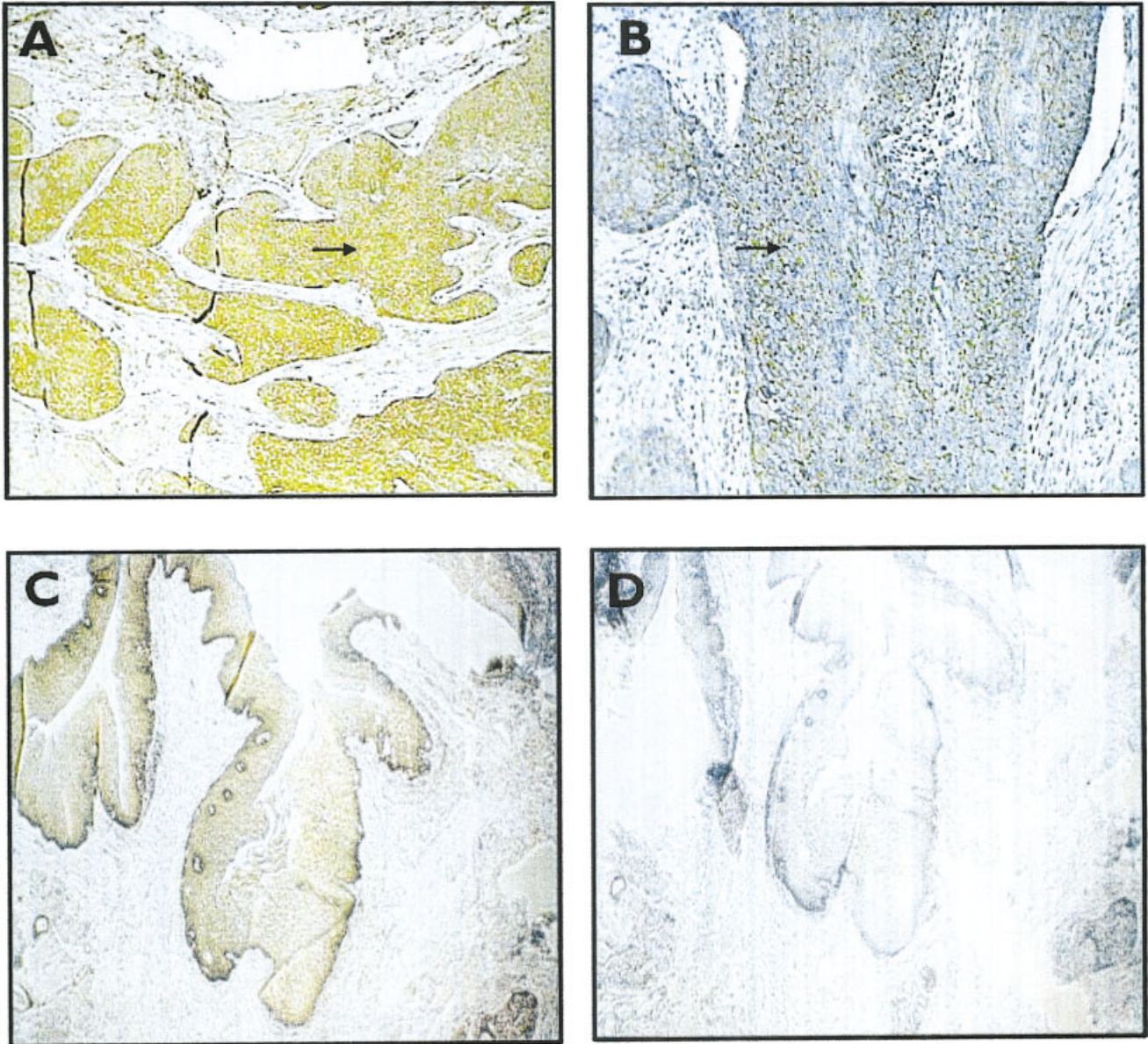


FIGURE 2 – HNSCC tissue samples stained for FGFR4. The degree of staining was evaluated using the semiquantitative method as described. (a) FGFR4 immunoreactivity in a strongly stained tumor and (b) in a poorly stained carcinoma. The specificity of the primary antibody was determined by preincubation with (d) or without (c) a blocking peptide (original magnification, $\times 20$, $\times 40$).

TABLE I – IMMUNOREACTIVITY OF FGFR4 IN 104 PATIENTS WITH HNSCC

	Expression		
	Low	Intermediate	High
Number of cases (%)	28 (27)	59 (57)	17 (16)

the oropharynx, we investigated the genotype distribution in a cohort of 104 patients to determine whether the FGFR4 glycine (Gly)/arginine (Arg) polymorphism plays a role in the progression of HNSCC. Because the polymorphism in codon 388 creates a new restriction site for the enzyme BstNI, genotyping was done by PCR-restriction fragment length polymorphism (RFLP). The overall frequency was 43% of homozygous Gly alleles, 43% of heterozygous Gly/Arg and 13% of homozygous mutant Arg alleles. The calculated allele frequencies (Gly, 65%; Arg, 35%) were

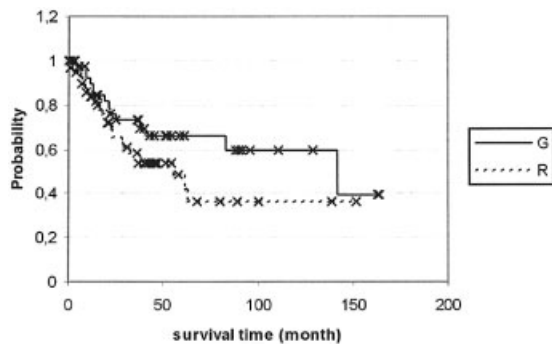
similar to those of 123 healthy control individuals and cancer patients described previously.¹⁸

Clinicopathologic parameters in oropharynx carcinomas

In order to investigate the hypothesis that FGFR4 Arg388 plays a role in the progression of oropharynx and oral cavity tumors, we examined the data for association between the FGFR4 genotypes and parameters known to influence clinical outcome. No correlation was found when comparing the FGFR4 genotype or expression level of all 104 patients with pathologic parameters such as age of diagnosis, tumor stage and histologic grading (Table II). Furthermore, allele distribution was not different in males and females or dependent on FGFR4 expression levels. Next, we investigated the impact of the FGFR4 Arg388 allele on the overall survival in our cohort of 104 patients in which 40 died within the follow-up period. Kaplan-Meier survival analysis of the squamous cell carcinoma patients stratified for the FGFR4 allele distribution

TABLE II – ASSOCIATION, BETWEEN FGFR4 ALLELES AND CLINICOPATHOLOGICAL VARIABLES IN PATIENTS WITH HNSCC

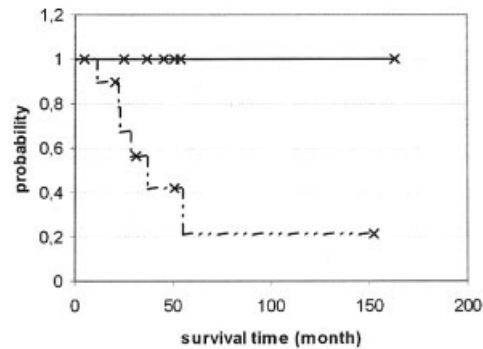
FGFR4 allele	Oral cavity carcinoma		
	Gly/Gly	Gly/Arg	Arg/Arg
Median age (years)	56	57	54
Sex			
M	36 (80%)	35 (76%)	13 (100%)
F	9 (20%)	11 (24%)	
Stage			
I	6 (13%)	7 (15%)	1 (8%)
II	7 (16%)	3 (7%)	2 (15%)
III	7 (16%)	9 (20%)	2 (15%)
IVA	19 (42%)	26 (57%)	8 (61%)
IVB	6 (13%)	1 (2%)	
Grade			
1	1 (2%)	2 (4%)	
2	25 (56%)	22 (48%)	4 (31%)
3	19 (42%)	22 (48%)	9 (69%)
FGFR4 expression			
Low	10 (22%)	12 (26%)	6 (46%)
Intermediate	28 (62%)	25 (54%)	6 (46%)
High	7 (16%)	9 (20%)	1 (8%)

**FIGURE 3** – Survival of patients with HNSCC according to the FGFR4 genotype. Kaplan-Meier analysis and the log-rank test were used for the association between FGFR4 genotypes and overall survival in patients with HNSCC ($n = 104$). G, FGFR4 Gly388; R, FGFR4 Arg388 (Gly/Arg and Arg/Arg).

yielded no clear-cut result for a follow-up time of 180 months, but showed a trend to a reduced overall survival of patients with the Arg388 allele (Fig. 3). In order to investigate whether a subgroup in our cohort of HNSCC patients exists that is particularly susceptible to FGFR4 Arg388, we grouped the patients according to their level of FGFR4 expression and analyzed the correlation between clinical parameters of patients with high FGFR4 expression and the FGFR4 genotype. Survival analysis showed that in the group of tumors with high FGFR4 expression ($n = 17$), patients with FGFR4 Arg388 have a reduced overall survival in comparison to patients with the Gly388 allele ($p = 0.032$; Fig. 4). Interestingly, only patients with FGFR4 Arg388 died in the follow-up period. Furthermore, comparing the genotype with the histologic stage of the tumors, we found that in the group with high FGFR4 expression, all patients with the Arg388 allele have an advanced tumor stage ($p = 0.023$), consisting of stages III, IVA and IVB (Table III). In summary, our data strongly support the conclusion that high expression of FGFR4 in connection with the Arg388 genotype is a prognostic parameter for HNSCC patients.

DISCUSSION

HNSCC is the sixth most frequent cancer worldwide, comprising almost 50% of all malignancies in some developing nations. Differences between normal epithelium and cancer cells of the

**FIGURE 4** – Viability of patients with high FGFR4 expression. The FGFR4 Arg388 allele is associated with a reduced overall survival time in patients with high FGFR4 expression. G, FGFR4 Gly388; R, FGFR4 Arg388 (Gly/Arg and Arg/Arg).**TABLE III** – CORRELATION OF THE FGFR4 GENOTYPE WITH THE HISTOLOGICAL STAGE OF HNSCC TUMORS

Genotype	Gly388 ($n = 7$)	Arg388 ($n = 10$)
Stage I and II	3	0
Stage III, IVA and IVB	4	10

$p = 0.0225$.

upper aerodigestive tract arise from specific alterations in genes controlling DNA repair, proliferation, apoptosis and angiogenesis. These genes include both tumor suppressors and oncogenes encoding growth factor receptors, signal transducers and transcription factors that regulate a wide variety of intracellular signaling pathways.²¹ For the FGFR family and their more than 20 known ligands, it was shown before that they play a critical role in cancer development. For example, high expression levels of FGF2, a well-characterized angiogenic factor, but also FGF1, FGFR1 and FGFR2, have been linked to the progression of HNSCC.^{22–24} However, a role for FGFR4 in this type of tumor has not yet been determined.

Here, we report that the FGFR4 is expressed at different levels, ranging from low or medium to strong, as determined by immunohistochemistry in all investigated tumors ($n = 104$) of the oral cavity. These results are compatible with previous studies demonstrating high FGFR4 expression in pancreatic cancer,²⁵ breast cancer^{19,26} and renal cell carcinoma²⁷ and suggest a role for FGFR4 in this malignancy. However, the comparison of FGFR4 expression with various prognostic parameter did not yield in significant findings. It seems that FGFR4 expression alone has no impact on disease progression in HNSCC. This speaks for the fact that indeed other factors, as for example genetic alterations in receptor tyrosine kinases, are key player in the progressive course of the disease. In fact, 2 recent studies have demonstrated that a polymorphism in the transmembrane domain of the FGFR4 is associated with poor prognosis in breast and colon and high-grade soft-tissue sarcoma.^{18,28} We used PCR-RFLP to characterize the incidence of the Arg388 allele in order to examine whether the FGFR4 polymorphism can control the outcome of the patients in our cohort. The allele distribution was similar to the previously reported frequency of the Arg388 allele in breast and colon cancer and also in healthy individuals.¹⁸ The correlation between the FGFR4 genotype and the viability of the patients revealed no clear-cut result, but showed a trend to a reduced overall survival for patients of the FGFR4 Arg388 isotype. However, when comparing the overall survival time of carriers of FGFR4 Arg388 and high protein expression, we found it to be significantly reduced in comparison to patients with the Gly388 allele and high FGFR4 expression. This findings were underlined by the observation that only patients with the FGFR4 Arg388 allele died in the follow-up

period. In addition, our study also shows that high-FGFR4-expressing patients with early-stage tumors (I and II) that have not spread to lymph nodes and have a high probability of survival predominantly express the Gly388 allele.

We conclude that expression of the wild-type FGFR4 (Gly388) is neutral to tumor progression or even might have a slight suppressive effect as reflected by the fact that all patients with high FGFR4 Gly388 protein expression have an improved clinical prognosis. However, expression of the FGFR4 Arg388 allele has the opposite effect since it impairs the tumor suppressor function and results in increased disease progression. Therefore, analysis of both FGFR4 expression and allele distribution is essential to improve the prediction of clinical prognosis and lead to new treatment strategies in patients with HNSCC.

Remarkably, Bange *et al.*¹⁸ demonstrated that the breast cancer cell line MDA-MB-231 infected with FGFR4 Gly388 showed a decreased motility in comparison to cells expressing the Arg allele *in vitro*. Furthermore, Cavallaro *et al.*²⁹ have recently described a

role for N-CAM-induced FGFR4 signaling in limiting the dissemination of metastatic β -cell tumors of the pancreas in mice. Interestingly, reduced N-CAM expression was associated with increased malignancy and poor prognosis in a variety of cancer types,³⁰ and Rip1Tag2 transgenic mice, which develop β -cell tumors in the pancreatic islets of Langerhans, show a marked increase in tumor metastases when crossed with N-CAM knockout mice.³¹ To our knowledge, we have shown for the first time that expression of a polymorphic RTK has the ability to influence tumor progression in squamous cell carcinoma of the oral cavity. Since RTKs and in particular FGFRs are a focus of molecular cancer therapy development, these results might open the door to new and advanced treatments of HNSCC.

ACKNOWLEDGEMENTS

The authors thank Norbert Prenzel and Andreas Gschwind for helpful discussions and critical reading of the manuscript.

REFERENCES

- Lipman SM, Hong WK. Second malignant tumors in head and neck squamous cell carcinoma: the overshadowing threat for patients with early-stage disease. *Int J Radiat Biol Phys* 1989;17:691-4.
- Strong MS, Incze J, Vaughan CW. Field cancerization in the aerodigestive tract: its etiology, manifestation, and significance. *J Otolaryngol* 1984;13:1-6.
- Kelloff GJ, Boone CW, Steele VK, Perloff M, Crowell J, Doody LA. Development of chemopreventive agents for lung an upper aerodigestive tract cancer. *J Cell Biochem* 1993;17F(Suppl):2-17.
- Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium: clinical implications of multicentric origin. *Cancer* 1953;6:963-8.
- Pringent SA, Lemoine NR. The type I (EGFR-related) family of growth factor receptors and their ligands. *Prog Growth Factor Res* 1992;4:1-24.
- Plowman GD, Green JM, Culouscou JM, Calton GW, Rothwell VM, Buckley S. Heregulin induces tyrosine phosphorylation of HER4/p180erbB4. *Nature* 1993;366:473-5.
- Tzahar E, Levkowitz G, Karunakaran D, Yi L, Peles E, Lavi S, Chang D, Liu N, Yayon A, Wen D. ErbB-3 and ErbB4 function as the respective low and high affinity receptors of all NDF/Heregulin isoforms. *J Biol Chem* 1994;269:25226-33.
- Sliwkowsky MX, Schaefer G, Akita RW, Lofgren JA, Fitzpatrick VD, Nuijens A, Fendly BM, Cerione RA, Vandlen RL, Carraway KL 3rd. Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptors for heregulin. *J Biol Chem* 1994;269:14661-5.
- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177-82.
- Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;244:707-12.
- Bange J, Zwick E, Ullrich A. Molecular targets for breast cancer therapy and prevention. *Nat Med* 2001;7:548-52.
- Bargman CI, Weinberg RA. Increased tyrosine kinase activity associated with the protein encoded by the activated neu oncogene. *Proc Natl Acad Sci USA* 1988;85:5394-8.
- Bellus GA, McIntosh I, Smith EA, Aylsworth AS, Kaitila I, Horton WA, Greenhaw GA, Hecht JT, Francomano CA. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet* 1995;10:357-9.
- Monsonego-Ornan E, Adar R, Feferman T, Segev O, Yayon A. The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation. *Mol Cell Biol* 2000;20:516-22.
- Schweitzer DN, Graham JM Jr, Lachman RS, Jabs EW, Okajima K, Przylepa KA, Shanske A, Chen K, Neidlich JA, Wilox WR. Subtle radiographic findings of achondroplasia in patients with Crouzon syndrome with acanthosis nigricans due to an Ala391Glu substitution in FGFR3. *Am J Med Genet* 2001;98:75-91.
- Capellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chopin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. *Nat Genet* 1999;23:18-20.
- Jeffers M, LaRochelle WJ, Lichtenstein HS. Fibroblast growth factors in cancers: therapeutic possibilities. *Expert Opin Ther Targets* 2002;6:469-82.
- Bange J, Prechtel D, Cheburkin Y, Specht K, Harbeck N, Schmitt M, Knyazeva T, Muller S, Gartner S, Sures I, Wang H, Imyanov E. Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. *Cancer Res* 2002;62:840-7.
- Lehtola L, Partanen J, Sistonen L, Korhonen J, Warri A, Harkonen P, Clarke R, Alitalo K. Analysis of tyrosine kinase mRNA including four FGF receptor mRNAs expressed in MCF-7 breast-cancer cells. *Int J Cancer* 1992;50:589-603.
- Hughes SE. Differential expression of the fibroblast growth factor receptor (FGFR) multigene family in normal human adult tissues. *J Histochem Cytochem* 1997;45:1005-19.
- Crowe DL, Hacia JG, Hsieh CL, Sinha UK, Rice H. Molecular pathology of head and neck cancer. *Histol Histopathol* 2002;17:909-14.
- Myoken Y, Myoken Y, Okamoto T, Sato JD, Takada K. Immunocytochemical localization of fibroblast growth factor-1 (FGF1) and FGF-2 in oral squamous cell carcinoma (SCC). *J Oral Pathol Med* 1994;23:451-6.
- Dellacono FR, Spiro J, Eisma R. Expression of basic fibroblast growth factor and its receptors by head and neck squamous carcinoma tumor and vascular endothelial cells. *Am J Surg* 1997;174:540-4.
- Janot F, El-Naggar AK, Morrison RS, Liu T, Taylor DL, Clayman GL. Expression of basic fibroblast growth factor in squamous cell carcinoma of the head and neck is associated with degree of histologic differentiation. *Int J Cancer* 1995;64:117-23.
- Leung HY, Gullick WJ, Lemoine NR. Expression and functional activity of fibroblast growth factors and their receptors in human pancreatic cancer. *Int J Cancer* 1994;59:667-75.
- Jaakkola S, Salmikangas P, Nylund S, Partanen J, Armstrong E, Pyrhonen S, Lehtovirta P, Nevanlinna H. Amplification of the *fgfr4* gene in human breast and gynaecological cancers. *Int J Cancer* 1993;54:378-82.
- Takahashi A, Sasaki H, Kim SJ, Kakizoe T, Miyao N, Sugimura T, Terada M, Tsukamoto T. Identification of receptor genes in renal cell carcinoma associated with angiogenesis by different hybridisation technique. *Biochem Biophys Res Commun* 1999;257:855-9.
- Morimoto Y, Ozaki T, Ouchida M, Umehara N, Ohata N, Yoshida A, Shimizu K, Inoue H. Single nucleotide polymorphism in fibroblast growth factor receptor 4 at codon 388 is associated with prognosis in high-grade soft tissue sarcoma. *Cancer* 2003;98:2245-50.
- Cavallaro U, Niedermeyer J, Fuxa M, Christofori G. N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. *Nat Cell Biol* 2001;3:650-7.
- Fogar P, Basso D, Pasquali C, De Paoli M, Sperti C, Roveroni G, Pedrazzoli S, Plebani M. Neural cell adhesion molecule (N-CAM) in gastrointestinal neoplasias. *Anticancer Res* 1997;17:1227-30.
- Hanahan D. Heritable formation of pancreatic β -cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 1985;315:115-22.