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The fibroblast growth factor receptor 4 (FGFR4) Arg^{388} allele correlates with survival in head and neck squamous cell carcinoma

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Abstract

Background: The increased expression of the fibroblast growth factor receptor 4 (FGFR4) has been identified in many human cancers. Recently, a single nucleotide polymorphism changing the sense codon 388 from glycine to arginine was identified in the *FGFR4* gene. The FGFR4 Arg^{388} allele was found to be associated with a poor prognosis for positive node breast cancer, high-grade soft-tissue sarcoma, colon carcinoma, and head and neck squamous cell carcinoma (HNSCC).
Methods: We decided to verify the impact of the FGFR4 Arg^{388} allele on survival as well as its association with histoclinical data in 75 cases of HNSCC. The FGFR4 Arg^{388} allele was detected by PCR-RFLP and DNA sequencing.
Results: The FGFR4 Arg^{388} allele was detected in 42.5% of the tumors (37% heterozygous Gly/Arg and 5.5% homozygous Arg/Arg). The presence of at least one Arg allele was significantly correlated with reduced overall survival after 24 months of follow-up. The cases involving the Arg allele presented an increased mortality risk of 2.2 if compared to the non-carrier cases.
*Conclusion:*The FGFR4 Arg^{388} allele is associated with a shortened survival.

Keywords: FGFR4; Head and neck squamous cell carcinoma; Prognosis; Polymorphism

Introduction

Head and neck squamous cell carcinoma (HNSCC) is an aggressive epithelial malignancy and is the sixth most common neoplasm in the world (Hasina et al., 2003). Epidemiological studies have shown that the incidence of oral cancer varies significantly across the continents, being more frequently diagnosed in developing countries (Gervasio et al., 2001). Sao Paulo City, the largest metropolitan area in Brazil, has one of the highest incidence of HNSCC in the world. As HNSCC is commonly diagnosed at advanced stages, this potentially curable disease is frequently fatal (Nagai et al., 1998).

Despite the advances in therapy, long-term survival of HNSCC patients has not improved significantly during the last 20 years. An important reason for this lack of progress is the relatively high recurrence rates. Local recurrences occur in about 10–30% of the cases involving advanced tumors even with histopathologically tumor-free surgical margins after resection (Tabor et al., 2001). For many years, this "field cancerization" concept has been used to explain the carcinogenetic effects of certain environmental agents, such as alcohol and tobacco, on upper aerodigestive tract by provoking genetic modifications in the whole epithelial linen (Braakhuis et al., 2003). Besides the large number of genes that might be involved in the progression of a precancerous cell into a cell with full malignant phenotype, it is currently accepted that some genes might be more important in enhancing the malignant transformation of these cells. The finding of such genes would improve the chances to develop better biomarkers and to enhance the

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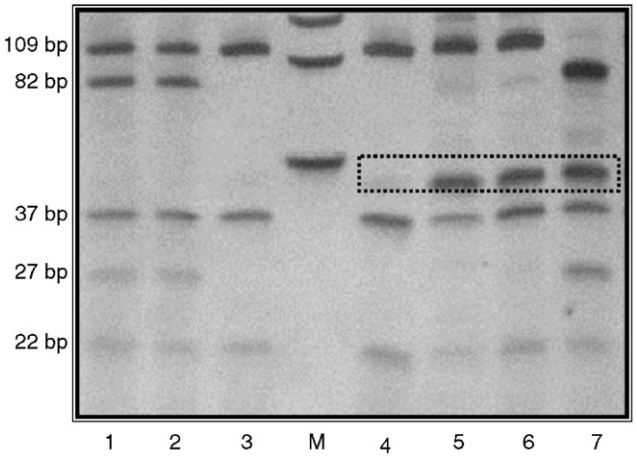

Fig. 1. Digestion of the 168-pb PRC fragment by using BstN1. Columns 1 and 2 show the digestion pattern of heterozygous Gly/Arg^{388} allele with fragments of 109, 82, 37, 27, and 22 pb. Columns 3, 4, 5, and 6 show the digestion pattern of homozygous Gly/Gly^{388} allele with fragments of 109, 37, and 22 bp. Column 7 shows the digestion pattern of homozygous Arg/Arg^{388} allele without 109 pb band. The dashed line means the primer dimmers. M refers to a 50-bp molecular marker.

target specificity of therapies, with the expectation of less collateral toxicity.

The tyrosine kinase (TK) family includes many growth factor receptors, cell cycle regulators, and oncoproteins (Meric et al., 2002). TK receptors (TKR) are receiving a great deal of attention due to their central role in cell growth and clear involvement in different types of cancers as well. TKR is a common name for a large group of different families of receptors, which also includes the fibroblast growth factor receptor (FGFR) family. The fibroblast growth factors compose a family of at least 24 related proteins with highly conserved amino acid sequences (Shimada et al., 2005). The FGFR is characterized by multiple extracellular immunoglobulin-like loops (ig-loops) maintained by disulfide bridges, an acid box between Ig-loops I and II, a single transmembrane domain, and a split intracellular tyrosine kinase domain. Four different high-affinity receptors (FGFR1, FGFR2, FGFR3, and FGFR4) can bind to the FGF ligands. Each FGFR subtype can also be differentially expressed to generate isoforms whose activation by distinct ligands results in different signaling specificities. Abnormal activation of FGFR can lead to autocrine growth stimulation and ultimately to cancer. Actually, specific FGFR genes aberrations such as translocations or point mutations are associated with multiple myeloma, leukemia, and many solid tumors (Xiao et al., 1998; Cappellen et al., 1999).

Single nucleotide polymorphisms (SNP) have been used to identify individual differences regarding the biological variances seen in clinical practice, such as drug sensitivity or immune response to malignant cells. The majority of SNP are silent but some are being implicated in the severity of disease presentation and progression. It has been recently reported that an SNP responsible for a missense mutation (G388R) of the *FGFR4* gene affects negatively the prognosis of breast cancer patients (Bange et al., 2002). The FGFR4 Gly388 allele is homozygous for G at position 1217, whereas the mutant allele is homozygous for A at the same position, thus changing codon 388 (GGG) to (AGG) and resulting in a prototype change from Gly to Arg in the FGFR4 transmembrane domain. Follow-up studies have found that the presence of this mutation was also overrepresented in worse prognosis cases of high-grade soft tissue sarcoma (Morimoto et al., 2003). FGFR4 hyper-expression (that might be due, at least in part, to the FGFR4 Gly^{388}Arg SNP) is also associated with both the worse prognosis in prostate cancer and the greater invasiveness of pituitary adenomas. However, recent reports have found non-significant relationship between FGFR4 Gly^{388}Arg SNP and the prognosis of breast and colon cancer (Jezequiel et al., 2004; Spinola et al., 2005).

To our knowledge, there is a single published report on prognostic significance of FGFR4 Gly^{388}Arg in human HNSCC. Streit et al. (2004) observed that high expression of FGFR4 in conjunction with Arg^{388} allele is associated with poor clinical outcome in these patients.

In the present study, we investigated the relationship between FGFR4 Arg^{388} allele and clinical outcome in patients with HNSCC by using a genetically distinct population in comparison to the one analyzed in the mentioned study by Streit et al.

Materials and methods

Tissue samples

Archival specimens from 75 cases of newly diagnosed HNSCC were obtained by surgical resection by the same surgical team during the period of 1996 to 2001 at the Sirio Libanes Hospital, Sao Paulo, Brazil. The specimens were fixed in formalin and embedded in paraffin. The age of the patients at the time of operation ranged from 22 to 89 years, with a median age of 59.8. The study included a total of 61 males and 14 females. Clinical staging was determined according to the UICC TNM Staging System, and histopathological grade was based on the WHO classification. The use of study specimens for the analyses was approved by the hospital research ethics committee.

DNA extraction

The DNA was purified by phenol chloroform extraction, followed by ethanol precipitation using 1 μg of glycogen as a carrier after digestion by proteinase K. Each paraffin section, 3 μm thick, was treated twice with xylene to remove the paraffin. Re-hydration of the sections was obtained by washing them consecutively in 100% ethanol, 75% (v/v) ethanol, 50% (v/v) ethanol, 25% (v/v) ethanol, and finally lysis buffer (10 mM Tris–HCL, pH 7.6, 1 mM EDTA and 0.6% SDS).

Table 1
Association of clinical and pathological variables with survival rate by univariate analysis (log rank test)

Variables	Significance (p value)
Surgical margin (close, insufficient, or sufficient)	0.0123
FGFR4 Gly/Gly^{388} versus FGFR4 −/Arg^{388} after 24-month follow-up	0.0213
N (histology)	0.0742
FGFR4 Gly/Gly^{388} versus FGFR4 −/Arg^{388}	0.2122
Gender	0.2160
Adjuvancy (radiotherapy, chemotherapy+radiotherapy, none)	0.4188
Staging (I, II, III, and IV)	0.5170
Primary tumor	0.6802
Degree of differentiation (high, moderate, low)	0.6940
Perineural invasion (with, without)	0.9204