http://pmjournal.ir Orginal Article

Summer 2021, Volume 6, Issue 22 (6-9)

Relationship between FGFR4 Gene rs351855 G/A Polymorphism and the Risk of Lung Cancer in the Northern Provinces

Ghadir A Jamal[1*], Hussam Saadi Aziz[2]

[1]Faculty of Allied Health Sciences, Departent of Medical Laboratory Sciences, Health Science Center, Kuwait University, Kuwait
[2]Southern Technical University College of Heaith and Medical

DOI: 10.22034/pmj.2021.246863

*Corresponding author: Ghadir A Jamal,Faculty of Allied Health Sciences, Departent of Medical Laboratory Sciences, Health Science Center, Kuwait University, Kuwait
. Email: Ghadir.jamal@ku.edu.kw

Submitted: 2021-07-21
Accepted: 2021-08-14

Keywords:
Lung cancer
Single Nucleotide Polymorphism
FGFR4
RFLP-PCR

Abstract

Lung cancer is the leading cause of cancer death in men and the second leading cause of cancer death in women worldwide. FGFR is involved in a variety of cellular processes including angiogenesis, wound healing, tissue repair, and tumorigenesis. Recently, a common polymorphism in the transmembrane domain of the FGFR4 gene, Gly388Arg, has been reported to correlate with alteration of cell migration in vitro and with disease progression and/or survival in breast, colon, prostate and lung cancer. To evaluate the prognostic significance of the FGFR4 Gly388Arg polymorphism in lung cancer, we analyzed a case-control study of 110 lung cancer patients and 90 healthy control. Genomic DNA from whole-blood specimens was extracted using Salting-out method. Quality of DNA was evaluated by electrophoresis. To determine the distribution of FGFR4 Arg388 and FGFR4 Gly388 alleles in lung carcinoma patients, RFLP-PCR was used. In this study demonstrated that there was no relationship between polymorphism of FGFR4 Gly388Arg gene and lung cancer. Also, no significant relationship was observed between this polymorphism and clinical and pathological features of patients. It is suggested that the large case-control studies are needed to detect genetic determinants affecting patients' prognosis, with the promise of targeting these putative genetic determinants to provide new therapeutic tools for patients with lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer morbidity and mortality in men, whereas, in women, it ranks third for incidence, after breast and colorectal cancer, and second for mortality, after breast cancer (1). Worldwide variation in the lung cancer burden and trends are primarily driven by historical differences in the uptake and reduction in tobacco use Lung cancer rates and trends vary substantially by sex, age, race/ethnicity, socioeconomic status, and geography because of differences in historical smoking patterns (2). the most important cause of lung cancer is exposure to tobacco smoke through active or passive smoking. tobacco smoke is a complex mixture of chemicals including multiple genotoxic lung carcinogens. Most cigarette smoke carcinogens are substrates for drug metabolizing enzymes such as the cytochromes P450, glutathione S-transferases, and UDP-glucuronosyl transferases which catalyze their conversion to more water soluble forms that are detoxified and can be readily excreted (3). But during this process, reactive intermediates such as carbocations or epoxides are produced and these electrophilic compounds can react with nucleophilic sites in DNA such as the nitrogen or oxygen atoms of deoxyguanosine and other DNA bases (4). The result is the formation of DNA adducts which are critical in the carcinogenic process. Everyone may have a unique combination of polymorphic traits that modify genetic susceptibility and response to drugs, chemicals and carcinogens (5). Developments in molecular biology have led to growing interest in investigation of biological markers, which may increase predisposition to lung carcinogenesis (6). Therefore, the high-risk genotype of an individual could be determined easily. As there are a large number of carcinogen-activating and -detoxifying enzymes, the variation in their expression and the complexity of exposures to tobacco carcinogens, the existence of multiple alleles at loci of those enzymes may result in differential susceptibilities of individuals (7). The genes encoding fibroblast growth factor receptors (FGFR) 1-4 are structurally related to receptor tyrosine kinases (RTK). FGFR are involved in

a variety of cellular processes including angiogenesis, wound healing, tissue repair, and tumorigenesis (8). Several aberrations and abnormalities in genes of FGFR family members, including point mutations, gene fusions, splice variations, and single nucleotide polymorphisms (SNP), have been the focus of studies in the past (9). Oncogenic effects of FGFR and their ligands, more than 20 in numbers, include initiation of DNA synthesis, enhancement of cell growth, invasion, and metastatic potential. Molecular abnormalities and overexpression of FGFR have been described (10).

Similar with other members of the FGFR family, aberrant FGFR4 activation is linked to the formation of tumors (11). Converging observations indicate that dysregulation of FGFR4 downstream signaling pathways, such as Wnt/_-catenin, JAK/STAT, and PI3K-AKT, leads to enhanced cell growth and metastatic potential in cancer progression. Recently, an explosion of investigations has revealed the associations of FGFR4 gene polymorphisms with the risk, prognosis, or treatment outcome of numerous cancer types, such as head and neck(12,13), lung, prostate, breast, colon, ovarian, liver , and uterine cervical cancer(12). Recently, a common polymorphism in the transmembrane domain of the FGFR4 gene, Gly388Arg, has been reported to correlate with alteration of cell migration in vitro and with disease progression and/or survival in breast, colon, prostate and lung cancer patients. However, an association between FGFR4 genotype and tumor aggressiveness or patients survival has not been confirmed in other cancers(13). Some of these studies were, however, conducted on relatively small sample sizes and subsequent studies of breast, colon, head and neck, and bladder cancers, have provided little support for an association between FGFR4 Gly388Arg genotype and prognosis (14). To evaluate the prognostic significance of the FGFR4 Gly388Arg polymorphism in lung cancer, we analyzed a case-control study of 110 lung cancer patients and 90 healthy control.

MATERIALS AND METHODS

This study included 110 patients with lung carcinoma at Northern province of Iran between 2018 and 2020. Each patient provided signed informed consent before initiation of the study. From all participants whole-blood specimens were collected for DNA extraction. The clinical information of the enrolled patients and the examined lifestyle variables (e.g., cigarette smoking) were obtained from medical records and questionnaires, respectively. Genomic DNA from whole-blood specimens was extracted using Salting-out method. quality of DNA was evaluated by electrophoresis. To determine the distribution of FGFR4 Arg388 and FGFR4 Gly388 alleles in lung carcinoma patients, RFLP-PCR was used, following primers were used: 5'-GAC CGCAGC AGC GCC CGA GGC CAG GTA TAC G– 3' (sense) and 5'–AGA GGG AAG CGG GAG AGC TTC TGC ACA GTG G–3' (antisense). G to A transition in codon 388 creates a new BstNI restriction site (New England Biolabs, Beverly, MA), which was used for discrimination of both alleles. PCR were applied in a 25 µL total PCR reaction volume and annealing temperature was 70°C. PCR products was digested with BstNI according to the manufacturer's instructions. Restriction fragments were analyzed on a 4% agarose gel. The Arg388 allele resulted in two distinctive fragments (80 bp, 29 bp) as opposed to a single distinctive band (109 bp) for the Gly388 allele. Statistical evaluations were performed using SPSS 19 (Chicago, IL). Frequencies of genotypes amongst different groups were calculated by the chi-quadrate test and P-values to assess the association between allele and malignancy.

RESULTS

In the present study, 110 lung carcinoma cases were recruited to explore the risk effect of FGFR4gene polymorphisms on the development of lung adenocarcinoma. Genotyping of the Gly388Arg polymorphism in all patients showed 59 (53.6%) were homozygous G/G alleles, 43 were heterozygous for the G/A alleles (39%) and 8 (7.4%) homozygous for the A/A alleles. control group include 90 healthy case and Genotyping all healthy case showed 41 (45.5%) were homozygous G/G alleles, 34 were heterozygous for the G/A alleles (37.7%) and 15 (16.6%) homozygous for the A/A alleles. in this study demonstrated that there was no relationship between polymorphism of FGFR4 Gly388Arg gene and lung cancer. Also, no significant relationship was observed between this polymorphism and clinical and pathological features of patients(Table1).

DISCUSSION

The American Cancer Society's estimates for lung cancer in the United States for 2021 are: About 235,760 new cases of lung cancer (119,100 in men and 116,660 in women) About 131,880 deaths from lung cancer (69,410 in men and 62,470 in women) (15). Gene changes related to lung cancer are usually acquired during a person's lifetime rather than inherited. Acquired mutations in lung cells often result from exposure to factors in the environment, such as cancer-causing chemicals in tobacco smoke, although a possible role for genetic susceptibility in the development of lung cancer has been inferred from familial clustering of the disease and segregation analyzes(16). Everyone may have a unique combination of polymorphic traits that modify genetic susceptibility and response to drugs, chemicals and carcinogens (17). Developments in molecular biology have led to growing interest in investigation of biological markers, which may increase predisposition to lung carcinogenesis. Therefore,

the high-risk genotype of an individual could be determined easily (18). As there are the great number of carcinogen-activating and -detoxifying enzymes, the variation in their expression and the complexity of exposures to tobacco carcinogens, the existence of multiple alleles at loci of those enzymes may result in differential susceptibilities of individuals (19).

In the present study, we evaluated association between FGFR4 SNPs rs351855 (Gly388Arg) with lung cancer. We found no significantly different frequencies of the FGFR4 rs351855 in patients with lung cancer and healthy cases.

Fibroblast growth factor receptors (FGFRs) have been found to play a vital role in tumorigenesis and cancer progression through increased cell proliferation, metastasis, and survival (20) .Compared with the other three FGFR family members, the signaling pathways and mechanisms of FGFR4 involved in cancer development are less characterized (21). The expression of FGFR4 is strictly regulated in human adult organs and tissues after fetal development, suggesting it perhaps has a particular relevance to tissue functions. Recently, elevated FGFR4 has been tightly correlated with cancer development and progression, making it an attractive target to develop novel and effective anticancer therapeutics. More efforts have been focused on developing selective inhibitors to target FGFR4, which show particular promise as an anticancer monotherapy or an adjunct treatment(22).

Several molecular alterations of FGFR4 leading to different gene variants have been identified. One of these variants, FGFR4–388Arg (rs351855 at the genotype level), harbors an amino acid substitution of an arginine for a glycine at codon 388. This FGFR4 variant correlates with disease progression and poorer prognosis in colon, prostate, head and neck, breast, and soft tissue tumors, among many others (23). In a similar study, Falvell et al. Examined the association between this polymorphism and the stage of the disease. The results of their study showed that there is a significant relationship between this polymorphism and the stage of lung cancer (24). In a similar study, Ali et al. examined the association between this polymorphism and the stage of the disease. The results of their study showed that there is a significant relationship between this polymorphism and the stage of lung cancer (25). In another study, Eva et al. examined the relationship between this polymorphism with FGFR4 gene expression and the occurrence of head and neck carcinoma. The results of their study showed that there is a significant relationship between this polymorphism and gene expression and the occurrence of head and neck carcinoma (26).

Nonetheless, the effects of this FGFR4 variant in NSCLC patient prognosis seem to be controversial. In a study involving Asian NSCLC patients, the FGFR4-388Arg variant correlated with poorer outcome in patients with lymph node involvement(27). However, contradictory results have been reported in other studies. In a work involving advanced NSCLC Asian patients, the FGFR4-388Arg variant correlated with better outcome, and in another study involving Caucasian NSCLC patients, no association between this FGFR4 variant and outcome was found. In some retrospective studies of lung cancer cohorts analyzing each histological subtype independently, the FGFR4-388Arg variant was linked to lymph node involvement and poorer overall survival (OS) in ADC patients (28). For SCC patients, however, association of this variant with prognosis has been described only in lymph node-involved patients.

Our demonstrations of no significant association of the FGFR4 Gly388Arg polymorphism with lung cancer and clinical and pathological features of patients. of northern province of Iran. it is suggested the large case-control studies are needed to detect genetic determinants affecting patients' prognosis, with the promise of targeting these putative genetic determinants to provide new therapeutic tools for patients with lung cancer.

REFERENCE

1.Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 2000;7:165–97.

2.Weinstein M, Xu X, Ohyama K, Deng CX. FGFR-3 and FGFR-4 function cooperatively to direct alveogenesis in the murine lung. Development 1998;125:3615–23.

3.Bange J, Prechtl D, Cheburkin Y, Specht K, Harbeck N, Schmitt M, Knyazeva T, Muller S, Gartner S, Sures I, Wang H, Imyanitov E, et al. Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. Cancer Res 2002;62:840–7.

4.Wang J, Stockton DW, Ittmann M. The fibroblast growth factor receptor-4 Arg388 allele is associated with prostate cancer initiation andprogression. Clin Cancer Res 2004;10:6169–78.

5.Spinola M, Leoni V, Pignatiello C, Conti B, Ravagnani F, Pastorino U, Dragani TA. Functional FGFR4 Gly388Arg polymorphism predicts prognosis in lung adenocarcinoma patients. J Clin Oncol 2005;23:7307–11.

6.Spinola M, Leoni VP, Tanuma J, Pettinicchio A, Frattini M, Signoroni S, Agresti R, Giovanazzi R, Pilotti S, Bertario L, Ravagnani F, Dragani TA. FGFR4 Gly388Arg polymorphism and prognosis of breast and colorectal cancer. Oncol Rep 2005;14:415–19.

7.Jezequel P, Campion L, Joalland MP, Millour M, Dravet F, Classe JM, Delecroix V, Deporte R, Fumoleau P, Ricolleau G. G388R mutation of the FGFR4 gene is not relevant to breast cancer prognosis. Br J Cancer 2004;90:189–93.

8.Matakidou A, El Galta R, Rudd MF, Webb EL, Bridle H, Eisen T, Houlston RS. Further observations on the relationship between the FGFR4 Gly388Arg polymorphism and lung cancer prognosis. Br J Cancer 2007;96:1904–7.

9.Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by

fibroblast growth factor receptors. Cytokine Growth Factor Rev 2005;16:139–49.

10.Colvin JS, White AC, Pratt SJ, Ornitz DM. Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. Development 2001;128:2095–106.

11.Usui H, Shibayama M, Ohbayashi N, Konishi M, Takada S, Itoh N. Fgf18 is required for embryonic lung alveolar development. Biochem Biophys Res Commun 2004;322:887–92.

12.Xie MH, Holcomb I, Deuel B, Dowd P, Huang A, Vagts A, Foster J, Liang J, Brush J, Gu Q, Hillan K, Goddard A, et al. FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4. Cytokine 1999;11:729–35. FIGURE 5 – Quantitation of FGFR4, FGF9 and FGF18 gene expression

in paired normal lung (n 5 19; open boxes) and lung ADCA (n

5 19; gray-filled boxes) tissue. Data are given as in Figure 4. For all three genes, differences between normal and tumor tissue were statistically significant at p < 1027. 2884 FALVELLA ET AL.

13.Zienolddiny S, Campa D, Lind H, Ryberg D, Skaug V, Stangeland LB, Canzian F, Haugen A. A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of non-small cell lung cancer in smokers. Carcinogenesis 2008;29:1164–9.

14.Weir BS. Genetic data analysis 2: methods for discrete population genetic data. Sunderlands: Sinauer Associates, Inc., 1996.

15.McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9:356–69.

16.Gianni-Barrera R, Gariboldi M, De Cecco L, Manenti G, Dragani TA. Specific gene expression profiles distinguish among functional allelic variants of the mouse Pthlh gene in transfected human cancer cells. Oncogene 2006;25:4501–4.

17.Sahadevan K, Darby S, Leung HY, Mathers ME, Robson CN, Gnanapragasam

VJ. Selective over-expression of fibroblast growth factor receptors 1 and 4 in clinical prostate cancer. J Pathol 2007;213:82–90.

18.Stadler CR, Knyazev P, Bange J, Ullrich A. FGFR4 GLY388 isotype suppresses motility of MDA-MB-231 breast cancer cells by EDG-2 gene repression. Cell Signal 2006;18:783–94.

19.Bailey JM, Singh PK, Hollingsworth MA. Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenic proteins. J Cell Biochem 2007;102:829–39.

20.Thiebault K, Mazelin L, Pays L, Llambi F, Joly MO, Scoazec JY, Saurin JC, Romeo G, Mehlen P. The netrin-1 receptors UNC5H are putative tumor suppressors controlling cell death commitment. Proc Natl Acad Sci USA 2003;100:4173–8

21.Yang, Y.; Zhou, Y.; Lu, M.; An, Y.; Li, R.; Chen, Y.; Lu, D.-R.; Jin, L.; Zhou,W.-P.; Qian, J.; et al. Associationmbetween fibroblast growth factor receptor 4 polymorphisms and risk of hepatocellular carcinoma. Mol. Carcinog. **2012**, 51, 515–521.

22.Chen, T.H.; Yang, S.F.; Liu, Y.F.; Lin, W.L.; Han, C.P.; Wang, P.H. Association of fibroblast growth factorreceptor 4 genetic polymorphisms with the development of uterine cervical cancer and patient prognosis. Reprod. Sci. **2018**, 25, 86–93.

23.Tsay, M.D.; Hsieh, M.J.; Lee, C.Y.; Wang, S.S.; Chen, C.S.; Hung, S.C.; Lin, C.Y.; Yang, S.F. Involvement of FGFR4 gene variants on the clinicopathological severity in urothelial cell carcinoma. Int. J. Environ. Res. Public Health **2019**, 17.

24.Tateno, T.; Asa, S.L.; Zheng, L.; Mayr, T.; Ullrich, A.; Ezzat, S. The FGFR4-g388r polymorphism promotes mitochondrial stat3 serine phosphorylation to facilitate pituitary growth hormone cell tumorigenesis. PLoS Genet. **2011**, 7, e1002400.

25.Ezzat, S.;Wang, R.; Pintilie, M.; Asa, S.L. FGFR4 polymorphic alleles modulate mitochondrial respiration: A novel target for somatostatin analog action in pituitary tumors. Oncotarget **2017**, 8, 3481 3494.

26.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, 2245–2250.

27.Dutra, R.L.; de Carvalho, M.B.; Dos Santos, M.; Mercante, A.M.; Gazito, D.; de Cicco, R.; Group, G.; Tajara, E.H.; Louro, I.D.; da Silva, A.M. FGFR4 profile as a prognostic marker in squamous cell carcinoma of the mouth and oropharynx. PLoS ONE **2012**, 7, e50747.

28.Choi, K.Y.; Rho, Y.S.; Kwon, K.H.; Chung, E.J.; Kim, J.H.; Park, I.S.; Lee, D.J. ECRG1 and FGFR4 single nucleotide polymorphism as predictive factors for nodal metastasis in oral squamous cell carcinoma. Cancer Biomark **2012**, 12, 115–124.