Genetics and Molecular Research

Link to retraction noticed

Correlation analysis between an IL-6 genetic polymorphism and non-small cell lung cancer prognosis

K. Zhao[1,2], J. Xu[3] and H. Tian[1]

[1]Department of Thoracic, Qilu Hospital of Shandong University, Shandong, China
[2]Department of Thoracic, Zibo Central Hospital, Zibo, Shandong, China
[3]Electric Power Hospital, Zibo, Shandong, China

Corresponding author: H. Tian
E-mail: fanrendeshiqq@126.com

Genet. Mol. Res. 15 (1): gmr.15017021
Received June , 201
Accepted December 3, 201
Published March , 20
DOI: http://dx.doi.org/10.4238/gmr.15017021

ABSTRACT. Interleukin-6 (IL-6) is a multifunctional cytokine that is involved in tumor cell proliferation, apoptosis, and differentiation. The purpose of this study was to evaluate the impact of the single nucleotide polymorphism (SNP) -174G/C in IL-6 on the prognosis and pain tolerance of non-small cell lung cancer (NSCLC) patients. DNA was extracted from the peripheral blood of 434 patients with NSCLC, which was diagnosed by cytology or histology. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the IL-6 -174G/C genotypes and their correlation with survival was analyzed. The IL-6 -174G/C genotypes were high IL-6 production type (G carriers - GG or GC genotypes) and low IL-6 production type (CC genotype). The correlation between the IL-6 SNP and pain level/analgesic use was also analyzed. Survival analysis showed that patients carrying the G allele (CG/GG) had a shorter survival time than patients with the CC genotype. The -174G/C SNP is in the promoter region of the IL-6 gene and may be associated with changes in gene transcription and serum cytokine levels. Presence of the IL-6 -174G/C SNP is significantly correlated with morphine equivalent daily dose. Patients

with the CC genotype needed a higher opioid dose than patients with the GG or GC genotypes. In conclusion, we found that the IL-6 -174G/C SNP is closely related to survival, analgesic use and pain tolerance in NSCLC patients. However, it is necessary to further validate the results with a larger patient cohort and elucidate the mechanisms of this SNP.

Key words: Interleukin-6; Small nucleotide polymorphism; Non-small cell lung cancer; Survival; Prognosis

INTRODUCTION

Lung cancer is a prevalent form of cancer that accounted for 26 and 29% of cancer deaths in women and men, respectively, in 2012. Eighty-five percent of these deaths were due to non-small cell lung cancer (NSCLC), a type of lung cancer with a particularly poor prognosis (Araújo et al., 2007; Jemal et al., 2011). Clinical and epidemiologic data show that inpatients diagnosed with NSCLC, 20% were associated with chronic infections, 30% were associated with smoking and pollutant inhalation (asbestos and silica), and 35% were related to diet (20% of which were due to a high-fat diet) (Molina et al., 2008). The lung cancer tumor microenvironment is composed of extracellular matrix, tumor cells, fibroblasts, inflammatory cells, cytokines, chemokines, hormones, and proteases. Chemokines are the basis of many biological processes and may play a role in the autocrine or paracrine function of tumor growth. Interleukin-6 (IL-6) is a multifunctional cytokine that is involved in tumor cell proliferation, apoptosis, and differentiation. It is expressed by malignant epithelial cells and thus is closely related to poor lung cancer prognosis (Goussetis and Wang, 2002; Lin and Karin, 2007; Aggarwal et al., 2009). The IL-6 gene is located at chromosome 7p21, and studies have found a single nucleotide polymorphism (SNP) in the promoter region. A point mutation from G to C at position -174 (rs1800795) could be correlated with changes in the IL-6 gene transcription rate, affecting IL-6 levels in the peripheral circulation. There are two primary genotypes formed by this SNP: the high IL-6 production type (GG and GC genotypes) and low IL-6 production type (CC genotype). Genetic studies have shown that the frequency of the G allele at position -174 has racial and ethnic differences (Brandao et al., 2012). The first goal of this study was to investigate the role of the -174G/C SNP in the IL-6 gene on NSCLC prognosis.

Pain is one of the most common and important factors affecting the prognosis of patients with NSCLC. It was reported that 30-40% of patients were in pain during the active period of the cancer, while this value reached 80% for patients in the later stages of disease. Opioids are the first choice for dealing with pain from cancer, but they have a significant difference in effect between individuals. High doses of opioids lead to neurotoxicity and repeated use may increase side effects and tolerance. Therefore, it is necessary to identify a new biomarker to reflect patient sensitivity to opioids. Cytokines may be involved in the mechanism for pain due to cancer and are related to opioid drug tolerance. During inflammation or neural injury, activated neurons release pro-inflammatory cytokines, leading to activation of pain transmission neurons. Synapses release numerous substances, including substance P and excitatory amino acids, which can aggravate the pain response. The second goal of this study was to investigate the relationship between the -174G/C SNP in the IL-6 gene and pain in NSCLC patients.

MATERIAL AND METHODS

Patient information

Between 1999 and 2012, 434 patients with NSCLC were enrolled in this study. The mean age of the selected patients was 64.0 ± 10.25 years. The inclusion criteria were: NSCLC diagnosed by histology or cytology with no anti-tumor therapy except to relieve symptoms; Eastern Cooperative Oncology Group score £2; and no other tumors present. Patients lacking tissue typing were excluded from the study. Smoking status was defined as the patient continuing to smoke after diagnosis. All patients provided written consent and the study was approved by the institutional review board in Zibo Central Hospital.

IL-6 -174G/C genotypes

DNA was extracted from white blood cells using the QIAmp® DNA Blood Mini Kit (Qiagen, USA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied to analyze the presence of the IL-6 -174G/C polymorphism. Five units of AvaIII (New England Biolabs, USA) was used to digest the PCR product at 37°C for 4 h. The restriction fragment was analyzed by electrophoresis on a 3% agarose gel. Fragments of 111 and 53 bp were consistent with the CC homozygous genotype, a 164-bp fragment represented the GG homozygous genotype, and all three fragments (52, 111 and 164 bp) represented the GC heterozygous genotype. Quality control was performed in 10% of samples and a negative control was used.

Cancer pain quantification and opioid us

Patients' treatments and follow-up information were collected to quantify pain after diagnosis. A score of 0 represented no pain and 10 represented intolerable pain. A record of opioid usage was also collected. Different types of opioid drugs were converted to morphine equivalent daily dose (MEDD) for comparison. Telephone follow-up was used when the medical record was incomplete.

Data processing

All statistical analyses were performed using the SPSS19.0 software (IBM SPSS, New York, NY, USA). The χ^2 or Fisher exact tests were applied to assess different proportions and the Kaplan-Meier curve was used to assess survival distribution. Lifetime was defined as the time span from diagnosis to death or last assessment time. Multivariate Cox proportional analysis was performed to determine the relationship between age, gender, tumor stage, pathology type, smoking, and IL-6 genotype with NSCLC overall survival. Hazard ratio (HRs) and 95% confidence interval (95%CI) were used to describe risk factors. Multivariate regression analysis was applied to evaluate the relationship between the IL-6 -174G/C genotype with cancer pain degree and opioid dosage. $P < 0.05$ was considered to be significant.

RESULTS

Patient information

Of the 434 enrolled NSCLC patients, 165 cases were epidermoid carcinoma, 207 were

adenocarcinoma, 40 were undifferentiated carcinoma, 14 were large cell carcinoma, 5 were mixed carcinoma, and 3 cases with unknown carcinoma. Of the patients, 78.11% were male and 23.43% were female, 72.12% were smokers; all data are summarized in Table 1. The frequency of genotypes GG, GC and CC was 37.6, 54.9, and 7.5%, respectively.

The IL-6 polymorphism genotypes were divided into two groups according to their functional activity: high IL-6 production group (G carriers- GC or GG genotypes) and low IL-6 production group (CC genotype). We found that the expression frequency of the G carrier genotypes was 92.5%. All genotypes showed no significant differences between patients' age at diagnosis (P = 0.367), tumor histology (P = 0.470), gender (P = 0.761), smoking history (P = 0.196), and tumor stage (P = 0.238).

Table 1. Patient information.

	Total of 434 cases	
	N	%
Gender		
Male	339	78.11
Female	93	21.43
Unknown	2	0.46
Age		
Mean ± SD	63.00 ± 10.25	
Histology		
Epidermoid carcinoma	165	38.02
Adenoma	207	47.70
Undifferentiated carcinoma	40	9.22
Large cell carcinoma	14	3.22
Mixed carcinoma	5	1.15
Unknown	3	0.69
Stage		
I	46	10.60
II	31	7.14
III	192	44.24
IV	162	37.33
Unknown	3	0.69
Smoking history		
Smoker	313	72.12
Non-smoker	114	26.27
Unknown	7	1.61

IL-6 -174G/C polymorphism and prognosis analysis

Kaplan-Meier analysis showed that the overall survival rate of NSCLC patients was different based on the presence of the IL-6 -174G/C polymorphism. The survival time of patients carrying the G allele (CG/GG) was significantly shorter than patients with the CC genotype (42.31 *vs* 62.79 months, P = 0.032) (Figure 1).

To further investigate the role of the IL-6 -174G/C polymorphism in overall survival rate,

the Cox proportional hazards model was applied to analyze gender, age, tumor stage, histology type, smoking history, and other related variables. It was found that tumor stage (P < 0.001), smoking history (P = 0.013), and IL-6 -174G/C polymorphism (P = 0.022) were independent prognostic factors for the overall survival rate of NSCLC patients (Table 2).

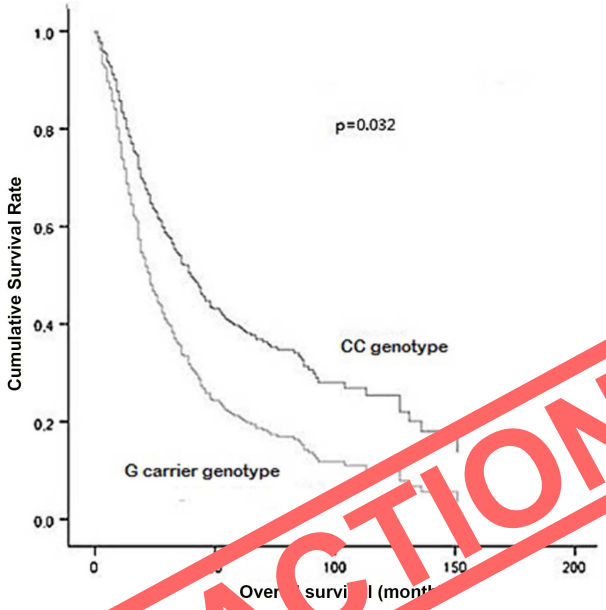

Figure 1. Kaplan-Meier survival curves based on IL-6 genotype. Survival curves are shown for NSCLC patients with the CC genotype and G carrier (GC or GG genotypes).

Table 2. Multivariate Cox regression analysis of overall survival rate.

	(hazard ratio)	95% CI	P value
IL-6 -174G	1.682	1.077-2.628	0.022
Gender	0.964	0.668-1.391	0.844
Age (≥63, <63	0.982	0.788-1.224	0.871
Smoking history	1.423	1.001-2.024	0.049
Histology type	0.933	0.810-1.074	0.336
Tumor stage	2.470	1.969-3.099	<0.001

Correlation between IL-6 -174G/C polymorphism and pain

On a scale of 1 to 10, a score greater than 7 was defined as severe pain. Our survey results revealed that 41% of patients presented severe pain at diagnosis and the average MEDD was greater than 120 mg/24h. Univariate analysis showed that the IL-6 -174G/C gene polymorphism was significantly correlated with MEDD (GG = 69.61; GC = 93.6; CC = 181.67; P = 0.004). The opioid dose in relation to the different IL-6 genotypes was significantly higher in males than females. Multivariate logistic regression analyses also showed that patients with the homozygous CC genotype needed a higher opioid dose than those with GG or GC genotypes (odds ratio = 4.7; 95% CI = 1.2-15.0). This data is summarized in Table 3.

Table 3. Correlation of IL-6 -174G/C gene polymorphism with pain level and MEDD (morphine equivalent daily dose).

Genotype	Pain level			MEDD		
	Total number	Male	Female	Total	Male	Female
	Mean value (SD)	Mean value (SD)	Mean value (SD)	Mean value (SD)	Mean value (SD)	Mean value (SD)
GG	5.00 (3.20)	4.68 (3.21)	5.40 (3.21)	69.61 (77.4)	72.89 (83.92)	64.81 (68.06)
GC	4.30 (2.81)	5.43 (3.18)	3.47 (2.24)	73.17 (93.6)	75.56 (117.90)	71.30 (71.97)
CC	3.42 (1.86)	3.67 (2.5)	3.40 (0.894)	181.67 (228.0)	235.71 (107.39)	106.00 (98.38)
GC + CC	4.11 (2.98)	4.9 (3.04)	3.46 (2.02)	97.7 (140.0)	120.40 (188.19)	77.5 (76.40)

DISCUSSION

Recent research has shown that inflammatory mediators may promote the development of a variety of tumors in cancers, including lung cancer. It was reported that the -174G/C polymorphism in IL-6 is correlated with lung cancer (Crohns et al., 2010). This study aimed to explore the link between the IL-6 -174G/C polymorphism and prognosis of patients with NSCLC. IL-6 plays an important role in tumor cells as a cytokine and is involved in many metabolic processes, such as malignant tumor cell differentiation, tumor growth, and regulation of the immune environment. It can also promote angiogenesis, inhibit tumor cell apoptosis, and mediate cell resistance. IL-6 directly stimulates tumor growth through activating several signaling pathways, such as the Ras/Raf/Mitogen-activated protein kinase/extracellular-signal-regulated kinase 1/2 signaling pathway (Naka et al., 2002; Ara and Declerck, 2010). Additionally, it can promote cell cycle activation by up-regulating cyclin D1, cyclin D2, cyclin B1, and Myc proteins through activation of signal transducer and activator of transcription 3 (STAT3) and down-regulation of the cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1}$ (Giri et al., 2001; Tsukasaki et al., 2007).

IL-6 is highly expressed in lung, brain, and liver tissues, and it promotes tumor metastasis by leading tumor cells into the peripheral circulation to enter in other organs (Culig et al., 2005). In recent years, it has been confirmed that IL-6 and IL-8 may recruit circulating tumor cells back to the original tumor location, which is known as "tumor self-cultivation", and this can accelerate tumor growth, neovascularization and stromal cell recruitment (Hefler et al., 2003; Zhang et al., 2009; Giannitrapani et al., 2006). Many studies have also suggested that high serum IL-6 levels are related to poor prognosis in breast, prostate, pancreatic, and ovarian cancers. Crohns et al. (2010) showed that levels of IL-6 and other cytokines may be involved in prognosis in lung cancer patients. Enewold et al. (2009) hypothesized that high serum levels of IL-6, IL-10, IL-12, and TNF-α are correlated with poor prognosis of lung cancer in African Americans and Caucasians. Thus, it can be deduced that high IL-6 levels in the peripheral circulation is related to a worse response to chemotherapy and poor clinical prognosis in NSCLC patients (Liu et al., 2012; Zarogoulidis et al., 2013). SNPs located in the promoter region of IL-6 can alter gene transcription, thereby affecting the serum level of the cytokine. A cytosine-to-guanine mutation in IL-6 at position -174 has been identified and the G allele has been confirmed to be related to multiple diseases, including cancer (Pine et al., 2011)

Several studies have indicated that IL-6 level is related to lung cancer prognosis (Cox et al., 2001). However, to our knowledge, this is the first study to explore the association between the IL-6 -174G/C gene polymorphism in the promoter region and NSCLC prognosis. Our results show that NSCLC patients with a G carrier genotype (GG or CG) had a significantly shorter survival time compared to those with the CC genotype (survival was ~20 months shorter; P = 0.032). Multivariate Cox proportional analysis revealed that the SNP plays an important role in NSCLC patient prog-

nosis (HR = 1.680; 95% CI = 1.075-2.624; P = 0.023). Another possible explanation is that IL-6 can regulate cytochrome P450 (CYP) enzyme expression and it has been confirmed that CYP1B1 is overexpressed in lung, colon, breast and other cancers. CYP1A1 and 1B1 can activate many pro-cancer substances, such as heterocyclic amines and polycyclic aromatic hydrocarbons. Patel et al. (2014) confirmed that IL-6 can inhibit microRNA 27b (miR27b) expression and upregulate CYP1B1 expression. IL-6 can induce phenotypic change in cells in colon tumors, which leads to drug resistance. It can also stimulate carcinogen metabolism *in situ*, leading to DNA damage and enhanced tumor invasiveness (Meenagh et al., 2002; Yang et al., 2014).

Preclinical data suggest that different IL-6 genotypes may affect opioid analgesic dose in pain management in NSCLC patients. Bianchi et al. (1999) revealed that the analgesic effect was reduced in IL-6 knockout mice, and IL-6 deficient mice produced tolerance to analgesic drugs. Our data also indicate a correlation between IL-6 genotype and pain in NSCLC patients. Serum IL-6 level was decreased in patients with the homozygous CC genotype, and they required a significantly higher opioid dose than patients with other genotypes (GG or GC). In addition, male NSCLC patients with the CC genotype required the highest dose of analgesic drugs. Thus, detecting IL-6 gene polymorphisms in NSCLC patients may predict patient response to opioid analgesics, which can help improve quality of life and prognosis.

In summary, the IL-6 -174G/C polymorphism is closely correlated with cancer pain, analgesic use, and survival in NSCLC patients. Further investigation is needed to elucidate the potential mechanism of this SNP and it is necessary to confirm these results through more exhaustive study of NSCLC patients.

REFERENCES

Aggarwal BB, Vijayalekshmi [illegible] and Sung B (2009). Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin. Cancer Res.* 15: 425-430. http://dx.doi.org/10.1158/1078-0432.CCR-08-0149

Ara T and Declerck YA (2010). Interleukin-6 in bone metastasis and cancer progression. *Eur. J. Cancer* 46: 1223-1231. http://dx.doi.org/10.1016/j.ejca.2010.01.026

Araújo A, Pinheiro A, Azevedo I, Coelho A, et al. (2007). Genetic polymorphisms of the epidermal growth factor and related receptor in non-small cell lung cancer--a review of the literature. *Oncologist* 12: 201-210. http://dx.doi.org/10.1634/theoncologist.12-2-201

Bianchi M, Maggi R, Pampinelli F, Rubino T, et al. (1999). Presence of a reduced opioid response in interleukin-6 knock out mice. *Eur. J. Neurosci.* 11: 1501-1507. http://dx.doi.org/10.1046/j.1460-9568.1999.00563.x

Brandao GD, Brega EF and Spatz A (2012). The role of molecular pathology in non-small-cell lung carcinoma-now and in the future. *Curr. Oncol.* 19 (Suppl 1): S24-S32. http://dx.doi.org/10.3747/co.19.1058

Coussens LM and Werb Z (2002). Inflammation and cancer. *Nature* 420: 860-867. http://dx.doi.org/10.1038/nature01322

Cox ED, Hoffmann SC, DiMercurio BS, Wesley RA, et al. (2001). Cytokine polymorphic analyses indicate ethnic differences in the allelic distribution of interleukin-2 and interleukin-6. *Transplantation* 72: 720-726. http://dx.doi.org/10.1097/00007890-200108270-00027

Crohns M, Saarelainen S, Laine S, Poussa T, et al. (2010). Cytokines in bronchoalveolar lavage fluid and serum of lung cancer patients during radiotherapy - Association of interleukin-8 and VEGF with survival. *Cytokine* 50: 30-36. http://dx.doi.org/10.1016/j.cyto.2009.11.017

Culig Z, Steiner H, Bartsch G and Hobisch A (2005). Interleukin-6 regulation of prostate cancer cell growth. *J. Cell. Biochem.* 95: 497-505. http://dx.doi.org/10.1002/jcb.20477

Enewold L, Mechanic LE, Bowman ED, Zheng YL, et al. (2009). Serum concentrations of cytokines and lung cancer survival in African Americans and Caucasians. *Cancer Epidemiol. Biomarkers Prev.* 18: 215-222. http://dx.doi.org/10.1158/1055-9965.EPI-08-0705

Giannitrapani L, Soresi M, Balasus D, Licata A, et al. (2013). Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. *World J. Gastroenterol.* 19: 2449-2455. http://dx.doi.org/10.3748/wjg.v19.i16.2449

Giri D, Ozen M and Ittmann M (2001). Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am. J. Pathol.* 159: 2159-2165. http://dx.doi.org/10.1016/S0002-9440(10)63067-2

Hefler LA, Grimm C, Ackermann S, Malur S, et al. (2003). An interleukin-6 gene promoter polymorphism influences the biological phenotype of ovarian cancer. *Cancer Res.* 63: 3066-3068.

Jemal A, Bray F, Center MM, Ferlay J, et al. (2011). Global cancer statistics. *CA Cancer J. Clin.* 61: 69-90. http://dx.doi.org/10.3322/caac.20107

Lin WW and Karin M (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. *J. Clin. Invest.* 117: 1175-1183. http://dx.doi.org/10.1172/JCI31537

Liu RY, Song X, Chen P, Lei Z, et al. (2012). Association between IL6 -174G/C and cancer: A meta-analysis of 105,482 individuals. *Exp. Ther. Med.* 3: 655-664.

Łukaszewicz M, Mroczko B and Szmitkowski M (2007). [Clinical significance of interleukin-6 (IL-6) as a prognostic factor of cancer disease]. *Pol. Arch. Med. Wewn.* 117: 247-251.

Meenagh A, Williams F, Ross OA, Patterson C, et al. (2002). Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. *Hum. Immunol.* 63: 1055-1061. http://dx.doi.org/10.1016/S0198-8859(02)00440-8

Molina JR, Yang P, Cassivi SD, Schild SE, et al. (2008). Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin. Proc.* 83: 584-594. http://dx.doi.org/10.1016/S0025-6196(11)60735-0

Naka T, Nishimoto N and Kishimoto T (2002). The paradigm of IL-6: from basic science to medicine. *Arthritis Res.* 4 (Suppl 3): S233-S242. http://dx.doi.org/10.1186/ar565

Patel SA, Bhambra U, Charalambous MP, David RM, et al. (2014). Interleukin-6 mediated up[illegible]lation of CYP1B1 and CYP2E1 in colorectal cancer involves DNA methylation, miR27b and STAT3. *Br. J. Cancer* 111: 128[illegible]96. http://dx.doi.org/10.1038/bjc.2014.540

Pine SR, Mechanic LE, Enewold L, Chaturvedi AK, et al. (2011). Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer. *J. Natl. Cancer Inst.* 103: 1112[illegible]1122. http://doi.org/10.1093/jnci/djr216

Yang M, Li C and Li M (2014). Association of interleukin-6 (-174 G/C) polymorphism with the prostate cancer risk: A meta-analysis. *Biomed. Rep.* 2: 637-643.

Zarogoulidis P, Yarmus L, Darwiche K, Walter R, et al. (2[illegible]). Interleukin-[illegible] cytokine: a multifunctional glycoprotein for cancer. *Immunome Res.* 9: 16535.

Zhang X, Yin P, Di D, Luo G, et al. (2009). [illegible] regulates MMP-10 expression via JAK2/STAT3 signaling pathway in a human lung adenocarcinoma cell line. *Anticancer Res.* 29: 4[illegible]97-45[illegible].