ORIGINAL ARTICLE
Oncology & Hematology

JKMS

DOI: 10.3346/jkms.2010.25.7.1024 • J Korean Med Sci 2010; 25: 1024-1028

Cox-2 and *IL-10* Polymorphisms and Association with Squamous Cell Carcinoma of The Head and Neck in a Korean Sample

Seung Won Jeong[1], Kyung Tae[1], Seung Hwan Lee[1], Kyung Rae Kim[1], Chul Won Park[1], Byung Lae Park[2], and Hyoung Doo Shin[2]

Department of Otolaryngology-Head and Neck Surgery[1], School of Medicine, Hanyang University, Seoul; Department of Genetic Epidemiology[2], SNP Genetics, Inc., Seoul, Korea

Received: 13 July 2009
Accepted: 30 December 2009

Address for Correspondence:
Kyung Tae, M.D.
Department of Otolaryngology-Head and Neck Surgery, School of Medicine, Hanyang University, 345 Wangsimni-gil, Seongdong-gu, Seoul 133-792, Korea
Tel: 82.2-2290-8585, Fax: 82.2-2293-3335
E-mail: kytae@hanyang.ac.kr

Cyclooxygenase-2 (COX-2) is involved in inflammation and carcinogenesis. Interleukin-10 (IL-10) is also regarded as anti-inflammatory factors with the multi-functional ability to positively and negatively influence functional immunity and tumor development. Genetic polymorphisms of *COX-2* and *IL-10* might contribute to the development of squamous cell carcinoma of the head and neck (SCCHN). The purpose of this study was to evaluate the association of *COX-2* and *IL-10* single nucleotide polymorphisms (SNPs) with the risk of SCCHN in a Korean sample. We analyzed the *COX-2* SNPs, -1329A>G, +1266C>T, and +6365T>C, and the *IL-10* SNPs, -1082A>G, +920T>G, and +3917T>C, in 290 Korean SCCHN patients and 358 healthy controls. There was no significant association between the risk of SCCHN and the three *COX-2* or three *IL-10* SNPs. We analyzed three haplotypes (ht1, ht2, ht3) for *COX-2* and found that *COX-2* ht3+/+ was associated with a decreased risk of SCCHN in a Korean sample, compared with the *COX-2* ht3 -/- genotype (P=0.03). Two haplotypes (ht1, ht2) of *IL-10* were analyzed and there was no statistical significance in the distribution of haplotypes. Based on these results, the *COX-2* haplotype ht3 can be used as a molecular biomarker to predict low risk groups of SCCHN in a Korean sample.

Key Words: Carcinoma, Squamous Cell; Head and Neck Neoplasms; *Cyclooxygenase 2*; *Interleukin-10*; Polymorphism, Genetic; Polymorphism, Single Nucleotide

INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) is the fifth most common cancer worldwide and accounts for 3.5% of the cancers registered in Korea during 2001 (1). SCCHN is believed to be induced by environmental and host factors. The main causative environmental factors are tobacco or alcohol use, viral infection, nutritional deficit, mineral inhalation and history of radiation exposure (2, 3). Accumulating evidence has shown that genetic polymorphisms influence the risk of environmental carcinogenesis (4-6), and that genetic susceptibility plays an important role in the development of SCCHN. Therefore, it has been suggested that susceptibility to SCCHN may be due to genetic polymorphisms in genes controlling carcinogen metabolism, repair of DNA damage, or genes related to cancer development, such as various cytokine genes and cyclooxygenase-2 (*COX-2*) (7-11).

Cyclooxygenase (COX) is a key enzyme that mediates the conversion of free arachidonic acid to prostaglandin. COX consists of two isoforms, COX-1 and COX-2 (12). COX-1 is constitutively expressed in most tissues for physiologic homeostasis. In comparison, COX-2 is rapidly activated by growth factors and mitogen and plays an important role in inflammation and tumor development (13).

Interleukin-10 (IL-10) is an important immunoregulatory cytokine in humans that is secreted by helper T-cells and synthesized by activated macrophages (14). IL-10 has the inhibitory properties of inflammatory cytokines like interferon gamma, IL-2, and IL-3, which are produced by macrophages and helper T-cells (14). The functions of IL-10 in humans range from the anti-inflammatory activity associated with auto-immune disease to tumor development and growth, as demonstrated by reports of increased IL-10 levels in cancer patients (14, 15).

Recently, several polymorphisms in *COX-2* and *IL-10* have been found to be associated with inflammation and tumor development. It is also thought that these polymorphisms may cause the differential expression of these genes, resulting in susceptibility to cancer development. Many studies have linked *COX-2* and *IL-10* genetic polymorphisms with various types of carcinoma (16, 17), but the *COX-2* and *IL-10* genetic polymorphisms have not been studied in cases of SCCHN in a Korean sample. In this study, we investigated the frequencies of single nucleotide polymorphisms (SNPs) in *COX-2* and *IL-10* between SCCHN patients and controls in a Korean sample and evaluated the effect of the *COX-2* and *IL-10* polymorphisms on the risk of SCCHN.

pISSN 1011-8934
eISSN 1598-6357

MATERIALS AND METHODS

Study population

For our hospital-based case-control study, we recruited subjects from the Department of Otolaryngology, Hanyang University Hospital, Seoul, Korea from 1997 to 2004. The case group consisted of 290 cases with pathologically-verified SCCHN. The cases were newly-diagnosed SCCHN patients, with the following primary sites: larynx (n=148), oral cavity (n=69), oropharynx (n=39), hypopharynx (n=39) and other (n=4). For the control group, we enrolled 358 hospital-based chronic otitis media, chronic sinusitis, and chronic tonsillitis patients. Cases and controls with a history of previous malignant disease or a genetic disease were excluded. The mean age of the case group was 62.6 yr (range, 28-90), with 252 males and 38 females. The mean age of the control group was 38.8 yr (range, 21-76), with 339 males and 19 females. All patients and controls were of Korean ethnicity. All participants provided informed consent. The Institutional Review Board of College of Medicine, Hanyang University approved the study protocol (December 15, 2008). Peripheral blood specimens were collected from all the subjects and stored at -80°C before DNA isolation.

Genotyping

We performed DNA extraction from peripheral blood using the Wizard™ Genomic DNA purification kit (Promega, Madison, WI, USA). We analyzed three SNPs of *COX-2* (-1329A>G, +1266C>T, and +6365T>C) and three SNPs of *IL-10* (-1082A>G, +920T>G, and +3917T>C). We performed single base extension (SBE) for genetic analysis of all SNPs, except for *COX-2* +6365T>C. The polymerase chain reactions (PCR) included primers (1.25 pM each), 5 ng genomic DNA, 250 μM dNTPs and 0.15 U *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) and were run using the GeneAmp PCR System 9700 Thermocycler (Applied Biosystems). The primers used are listed in Table 1. Using the SNaPshot ddNTP primer extension kit (Applied Biosystems), we performed the primer extension reaction. To clean-up the primer extension reaction products, 1 U shrimp alkaline phosphatase (Amersham Life Science, Cleveland, Ohio, USA) was added to the reaction mixture, and the mixture was incubated at 37°C for one hour, followed by 15 min at 72°C for enzyme inactivation. We added the amplified material and Genescan 120Liz size-standard solution (Applied Biosystems) in Hi-Di formamide (Applied Biosystems) and incubated the reaction at 95°C for five minutes, followed by incubation on ice for five minutes. Electrophoresis was performed using the reaction mixture with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). We used ABI Prism GeneScan and Genotyper to perform the genetic analyses.

The analysis of the *COX-2* +6365T>C SNP was performed using the TaqMan assay. The forward and reverse primers were: 5'-GCATCTTCCATGATGCATTAGAAGTAAC-3', 5'-GCACTGATACCTGTTTTTGTTTGATGA-3'. The probe for the wildtype (T allele) was 5'-AAGTACTTTTGGTTATTTTT-3'. The 5' end was conjugated with VIC™ fluorescent dye and the 3' end was conjugated with TAMRA™ quencher dye. The probe for the mutant type (C allele) was 5'-ACTTTTGGTCATTTTT-3', with the 5' end bound to FAM™ fluorescent dye and the 3' end bound to TAMRA™ quencher dye. PCR was performed in a 5 μL reaction that consisted of a mixture of TaqMan universal PCR master mix (Applied Biosystems), UNG, primers (900 μM), probe (200 nM) and genomic DNA (20 μg). The PCR reaction conditions were as follows: 50°C for two minutes, 95°C for ten minutes and then 15 sec denaturing, final annealing and extension at 60°C for one minute, for 40 amplification cycles. The TaqMan assay plate was transferred to a Prism 9700HT instrument and genetic analysis was performed by measuring the fluorescence level in each well.

Table 1. Primer sequences for *COX-2* and *IL-10* SNP genotyping using the single-base extension method

Locus	Primer	Sequence (5′-3′)
COX-2 -1329A>G	forward	TCCTCCCTGAGCACTACCCAT
	reverse	CAGTTGCCTGGGCTTATTGG
	extension	GTTAAACAAAAGCAAAGATGAAATTCCA
COX-2 +1266C>T	forward	CTTCCTTCGAAATGCAATTATGAGT
	reverse	TGAGCAGACTGTATAAACTGGATGG
	extension	CATGTAAGTACAAGTGTCTTTCTAAGGTTTTTAGC
IL-10 -1082A>G	forward	CCCAACTGGCTCCCCTTAC
	reverse	GGAGGCTGGATAGGAGGTCC
	extension	ATGATGATACTTTCCTCTTACCTATCCCTACTTCCCC
IL-10 +920T>G	forward	TGCTTTCCCTTCAAAATCGG
	reverse	CCTTCATTTGCTGCAGGAAGA
	extension	ATGATTTTTTTGGGCCAGAGCCAATTT
IL-10 +3917T>C	forward	GAGACATCAGGGTGGCGACT
	reverse	GGCTCCCCAAAGAACATTTTT
	extension	GACCCAGCCCCTTGAGAAACCTTATTGTACCTCTCT

Statistical analyses

Statistical verification of the associations of *COX-2* and *IL-10* genetic polymorphisms with SCCHN and with the normal control group was performed using a chi square test. The odds ratios (OR) and 95% confidence intervals were obtained using a logistic regression model. All statistical analyses were performed using the SPSS statistical package (SPSS, version 12.0, Inc., Chicago, IL, USA).

RESULTS

COX-2 polymorphisms

For the *COX-2* -1329A>G SNP (genotyping was available in 621 of 648 subjects), the frequency of variant allele G was 44.2% and heterozygosity was 46.8%. The frequency of the *COX-2* genotypes, -1329 AA, AG and GG, were 31.7%, 46.9%, 21.4% in the case group and 32.9%, 46.9%, and 20.3% in the control group respectively. The odds ratio and respective 95% confidence in-

tervals for the genotype AG and GG in reference to AA were 1.08 (0.78-1.49) and 1.00 (0.61-1.65), respectively. For the *COX-2* +1266C>T SNP (genotyping was available in 621 of 648 subjects), the frequency of variant allele T was 0.7% and the heterozygosity was 1.4%. The frequency of the *COX-2* genotypes, +1266 CC, CT, and TT, were 97.8%, 1.8%, and 0.4% in the case group and 98.9%, 1.1%, and 0% in the control group, respectively. The relative risk of CT to CC was 1.11 (0.21-5.99). For the *COX-2* +6365T>C SNP (genotyping was available in 587 of 648 subjects), the frequency of variant allele C was 20.8% and heterozygosity was 33.2%. The frequency of the *COX-2* genotypes, +6365 TT, TC, and CC, were 64.7%, 32.1%, and 3.2% in the case group and 61.2%, 34%, and 4.7% in the control group, respectively. The relative risks of TC and CC to TT were 1.01 (0.55-1.84), and 0.78 (0.49-1.26), respectively. We found no statistical significance in our analyses (Table 2).

COX-2 haplotypes

We identified six haplotypes (GCT, ACT, ACC, ATC, GCC, GTT) for *COX-2* -1329A>G, +1266C>T, and +6365T>C and analyzed the three most common haplotypes, which we abbreviated as ht1 (GCT), ht2 (ACT), and ht3 (ACC) (Table 3). The haplotype distributions are summarized in Table 4.

The frequencies of ht3 -/-, +/-, and +/+ were 69.3%, 27.8%, 2.9% in the case group and 62.4%, 33.0%, and 4.6% in the control group, respectively. The relative risk of ht3 +/- to -/- was 1.05 (0.55-2.01) and the relative risk of ht3+/+ to -/- was 0.60 (0.38-0.96). So, compared with the ht3-/- genotype, the ht3+/+ genotype was significantly associated with a decreased risk of HNSCC in Koreans (P=0.03).

IL-10 polymorphisms

For the *IL-10* -1082A>G SNP (genotyping was available in 628 of 648 subjects), the frequency of the variant allele G was 7% and the heterozygosity was 13.2%. The frequencies of *IL-10* genotypes, -1082 AA, AG, and GG, were 85.6%, 13.7%, and 0.7% in the case group and 86.9%, 12.9%, and 0.3% in the control group, respectively. The relative risks of genotypes AG and GG to AA were 0.75 (0.40-1.43) and 1.03 (0.28-3.81), respectively. For the *IL-10* +920T>G SNP (genotyping was available in 609 of 648 subjects), the frequency of the variant allele G was 30.3% and the heterozygosity was 41.2%. The genotype frequencies of TT, TG and GG were 45.5%, 46.2%, and 8.3% in the case group and 51.6%, 37.4%, and 11.0% in the control group, respectively. The relative risks of TG and GG to TT were 1.36 (0.85-2.16) and 0.88 (0.59-1.30), respectively. For the *IL-10* +3917T>C SNP (genotype was available in 612 of 648 subjects), the variant allele C was 3.5% and the heterozygosity was 6.8%. The genotype frequencies of TT, TC, and

Table 3. The frequencies of *COX-2* gene haplotypes in Korean head and neck squamous cell carcinoma patients and normal control subjects

Haplotype	-1329A>G	+1266C>T	+6365T>C	Frequency
ht1	G	C	T	0.438
ht2	A	C	T	0.357
ht3	A	C	C	0.192
ht4	A	T	C	0.008
ht5	G	C	C	0.004
ht6	G	T	T	0.001

Ht, haplotype.

Table 2. Logistical analysis of the *COX-2* gene in Korean head and neck squamous cell carcinoma patients and normal control subjects

Gene	Loci	Genotype	Cancer	Normal	OR (95% CI)	*P*
COX-2		AA	86 (31.7%)	115 (32.8%)	1	
	-1329A>G	AG	127 (46.9%)	164 (46.9%)	1.08 (0.78-1.49)	0.64
		GG	58 (21.4%)	71 (20.3%)	1.00 (0.61-1.65)	0.99
		CC	266 (97.8%)	345 (98.9%)	1	
	+1266C>T	CT	5 (1.8%)	4 (1.1%)	1.11 (0.21-5.99)	0.99
		TT	1 (0.4%)	0 (0.0%)		0.90
		TT	161 (64.7%)	207 (61.3%)	1	
	+6365T>C	TC	80 (32.1%)	115 (34.0%)	1.01 (0.55-1.84)	0.98
		CC	8 (3.2%)	16 (4.7%)	0.78 (0.49-1.26)	0.31

OR, odds ratio; CI, confidence interval.

Table 4. Analysis of haplotypes in the *COX-2* gene in Korean head and neck squamous cell carcinoma patients and normal control subjects

Gene	Loci	Genotype	Cancer	Normal	OR (95% CI)	*P*
COX-2		-/-	89 (32.1%)	114 (32.5%)	1	
	ht1	ht1/-	129 (46.6%)	171 (48.7%)	0.91 (0.66-1.26)	0.56
		ht1/ht1	59 (21.3%)	66 (18.8%)	0.88 (0.54-1.43)	0.61
		-/-	113 (40.8%)	152 (43.3%)	1	
	ht2	ht2/-	124 (44.8%)	153 (43.6%)	0.32 (0.92-1.89)	0.13
		ht2/ht2	40 (14.4%)	46 (13.1%)	1.35 (0.86-2.13)	0.19
		-/-	192 (69.3%)	219 (62.4%)	1	
	ht3	ht3/-	77 (27.8%)	116 (33.0%)	0.15 (0.55-2.01)	0.89
		ht3/ht3	8 (2.9%)	16 (4.6%)	0.60 (0.38-0.96)	0.03

OR, odds ratio; CI, confidence interval; ht, haplotype.

DOI: 10.3346/jkms.2010.25.7.1024

Table 5. Logistical analysis of the *IL-10* gene in Korean head and neck squamous cell carcinoma patients and normal control subjects

Gene	Loci	Genotype	Cancer	Normal	OR (95% CI)	*P*
IL-10		AA	238 (85.6%)	304 (86.8%)	1	
	-1082A>G	AG	38 (13.7%)	45 (12.9%)	0.75 (0.40-1.43)	0.39
		GG	2 (0.7%)	1 (0.3%)	1.03 (0.28-3.81)	0.96
		TT	120 (45.5%)	178 (51.6%)	1	
	+920T>G	TG	122 (46.2%)	129 (37.4%)	1.36 (0.85-2.16)	0.20
		GG	22 (8.3%)	38 (11.0%)	0.88 (0.59-1.30)	0.51
		TT	247 (92.8%)	322 (93.1%)	1	
	+3917T>C	TC	18 (6.8%)	24 (6.9%)	0.71 (0.30-1.70)	0.44
		CC	1 (0.4%)	0 (0.0%)		0.99

OR, odds ratio; CI, confidence interval.

Table 6. Analysis of *IL-10* haplotypes in Korean head and neck squamous cell carcinoma patients and normal control subjects

Gene	Loci	Genotype	Cancer	Normal	OR (95% CI)	*P*
IL-10		ht1/ht1	117 (45.3%)	176 (51.8%)	1	
	ht1	ht1/-	121 (46.9%)	129 (37.9%)	1.38 (0.86-2.19)	0.18
		-/-	20 (7.8%)	35 (10.3%)	0.81 (0.53-1.23)	0.32
		-/-	239 (92.6%)	306 (90.0%)	1	
	ht2	ht2/-	9 (3.5%)	14 (4.1%)	0.65 (0.18-2.34)	0.51
		ht2/ht2	10 (3.9%)	20 (5.9%)	0.76 (0.46-1.26)	0.29

OR, odds ratio; CI, confidence interval; ht, haplotype.

CC were 92.9%, 6.8%, and 0.4% in the case group and 93.1%, 6.9%, and 0% in the control group, respectively. The relative risk of TC to TT was 0.71 (0.30-1.70). No significant statistical results were obtained in our analyses of *IL-10* polymorphisms (Table 5).

IL-10 haplotypes

Two haplotypes, ht1(ATT) and ht2(AGT) for the *IL-10* SNPs -1082A>G, +920T>G, and +3917T>C were analyzed. The haplotype distributions are summarized in Table 6. There was no significant difference in the distribution of the two *IL-10* haplotypes in the SCCHN patient and normal control groups.

DISCUSSION

Development of cancer is dependent on individual genetic susceptibility. For example, genetic polymorphisms, activation of cancer genes and alterations of the immune system are determinants of individual genetic susceptibility (18). The most common genetic polymorphism in the population is SNP, which are single nucleotide substitutions within genes. These small changes in DNA can cause significant alterations in normal genetic function, metabolism, repair of DNA, personal sensitivity and ultimately malignant tumor development (19).

COX-2 expression and activity is induced by growth factors, carcinogens and oncogenes (20). COX-2 overexpression and its association with cancer development have been reported especially in colorectal cancer and SCCHN (21). The *COX-2* gene is located at 1q25.2-q25.3 and is encoded by 10 exons (22). To date, 25 polymorphisms in the *COX2* gene have been identified in a Korean sample.

Our study analyzed the association of *COX-2* SNPs 1329A>G, +1266C>T, +6365T>C with SCCHN in a Korean sample. There was no definite statistical significance between cancer risk and genetic polymorphism for three *COX-2* SNPs. However, the frequency of the haplotype ht3 (ACC) differed significantly. Haplotype ht3 +/+ (ACC/ACC) was associated with a significantly decreased risk of SCCHN, compared with the ht3-/- group, suggesting a possible involvement of ht3 in the development of SCCHN. There has been no study that evaluates *COX-2* SNPs that were analyzed in our study in SCCHN. In a study that examined the association of *COX-2* -765 G>C polymorphism with the risks of oral squamous cell carcinoma in a Taiwan population, the authors reported that *COX-2* -765C allele was a protective factor against oral squamous cell carcinoma (23). However, in other study in the Netherlands, there was no risk-modifying effect of *COX-2* -1195 A>G and -765 G>C polymorphisms in head and neck carcinogenesis (24).

IL-10 is an important immune regulatory cytokine in humans that is mainly secreted by helper T-cells and synthesized by activated macrophages (14). The predominant effect of *IL-10* is reduced inflammation, and it contributes to the control of immune-related cellular proliferation and differentiation (14, 15).

The *IL-10* gene is located on chromosome 1q31-32 and is encoded in 5 exons. Numerous genetic polymorphisms in *IL-10* have been reported and nearly all of them are SNPs (25). In Italian patients with undifferentiated carcinoma of the nasopharynx, it has been reported that *IL-10* -1082A>G, -819C>T, and -592A>C SNPs were not associated with cancer development (26). In our study, we analyzed three *IL-10* SNPs and two haplotypes. The *IL-10* -1082A>G, +920T>G, and +3917T>C SNPs and their respective haplotypes were not associated with the risk of SCCHN in a Korean sample.

In conclusion, *COX-2* ht3+/+ was associated with a significantly decreased risk of SCCHN in a Korean sample, compared with the *COX-2* ht3-/- genotype. Based on this result, the *COX-2* haplotype ht3 can be used as a molecular biomarker to predict low risk groups of SCCHN in a Korean sample.

REFERENCES

1. Kim KM, Kim YM, Shim YS, Kim KH, Chang HS, Choi JO, Rho YS, Kim MS, Choi EC, Choi G, Sung MW, Kim SY, Lee YS, Baek JH, Kim SH, Kim YH, Im JH, Choi SH, Kim JH; Study Group of Korean Society of Head and Neck Surgeons. *Epidemiologic survey of head and neck cancers in Korean. J Korean Med Sci 2003; 18: 80-7.*
2. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF Jr. *Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988; 48: 3282-7.*
3. McKaig RG, Baric RS, Olshan AF. *Human papillomavirus and head and neck cancer: Epidemiology and molecular biology. Head Neck 1998; 20: 250-65.*
4. Zheng Y, Shen H, Sturgis EM, Wang LE, Shete S, Spitz MR, Wei Q. *Haplotypes of two variants in p16(CDKN2/MTS-1/INK4a) exon3 and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Epidemiol Biomarkers Prev 2002; 11: 640-5.*
5. Shen H, Zheng Y, Sturgis EM, Spitz MR, Wei Q. *P53 codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Lett 2002; 183: 123-30.*
6. Zheng Y, Li L, Shen H, Sturgis EM, Eicher SA, Strom SS, Spitz MR, Wei Q. *Polymorphism hCHK2/hCdsl codon 84 allele and risk of squamous cell carcinoma of the head and neck- a case-control analysis. Carcinogenesis 2001; 22: 2005-8.*
7. Yang M, Kim WH, Choi Y, Lee SH, Kim KR, Lee HS, Tae K. *Effects of ERCC1 expression in peripheral blood on the risk of head and neck cancer. Eur J Cancer Prev 2006; 15: 269-73.*
8. Yang M, Kang MJ, Choi Y, Kim CS, Lee SM, Park CW, Lee HS, Tae K. *Association between XPC expression, genotype, and the risk of head and neck cancer. Environ Mol Mutagen 2005; 45: 374-9.*
9. Tae K, Lee HS, Park BJ, Park CW, Kim KR, Cho HY, Kim LH, Park BL, Shin HD. *Association of DNA repair gene XRCC1 polymorphism with head and neck cancer in Korean population. Int J Cancer 2004; 111: 805-8.*
10. Shin CS, Ahn KS, Tae K, Lee HS, Kim HJ, Kong G. *Genetic susceptibilities of Cytochrome P4501A1 and Glutathione S-transferase M1 to the risk for Korean head and neck squamous cell carcinoma patients. Korean J Otolaryngol - Head Neck Surg 1999; 42: 202-8.*
11. Ko KM, Ahn KS, Tae K, Lee SH, Kong G. *Genetic polymorphism of Cytochrome P4501A1 Exon7 and Glutathione S-transferase M1 in the head and neck squamous cell carcinoma patients. Korean J Otolaryngol - Head Neck Surg 1999; 42: 1405-12.*
12. Ondrey FG. *Arachidonic acid metabolism: a primer for head and neck surgeons. Head Neck 1998; 20: 334-49.*
13. Tsujii M, DuBois RN. *Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995; 83: 493-501.*
14. Mocellin S, Marincola FM, Young HA. *Interleukin-10 and the immune response against cancer: a counterpoint. J Leukoc Biol 2005; 78: 1043-51.*
15. Howell WM, Rose-Zerilli MJ. *Interleukin-10 polymorphism, cancer susceptibility and prognosis. Fam Cancer 2006; 5: 143-9.*
16. Shahedi K, Lindstrom S, Zheng SL, Wiklund F, Adolfsson J, Sun J, Augustsson-Bälter K, Chang BL, Adami HO, Liu W, Grönberq H, Xu J. *Genetic variation in the COX-2 gene and the association with prostate cancer risk. Int J Cancer 2006; 119: 668-72.*
17. Howell WM, Turner SJ, Bateman AC, Theaker JM. *IL-10 promoter polymorphism influence tumour development in cutaneous malignant melanoma. Genes Immun 2001; 2: 25-31.*
18. De Andrade M, Amos CI, Foulkes WD. *Segregation analysis of squamous cell carcinoma of the head and neck: Evidence for a major gene determining risk. Ann Hum Genet 1998; 62: 505-10.*
19. Nussbaum RL. *Genetic variation in individuals: mutation and polymorphism. In: Nussbaum RL, Mcinnes RR, Willard HF. eds. Thompson & Thompson genetics in medicine. W.B.Saunders 2002; 79-94.*
20. Lands WE. *Biosynthesis of prostaglandins. Annu Rev Nutr 1991; 11: 41-60.*
21. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. *Up-regulation of cyclooxypgenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994; 107: 1183-8.*
22. Hedelin M, Chang ET, Wiklund F, Bellocco R, Klint A, Adolfsson J , Shahedi K, Xu J, Adami HO, Grönberg H, Bälter KA. *Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. Int J Cancer 2006; 120: 398-405.*
23. Lin YC, Huang HI, Wang LH, Tsai CC, Lung O, Dai CY, Yu ML, Ho CK, Chen CH. *Polymorphisms of COX-2 -765G>C and p53 codon 72 and risks of oral squamous cell carcinoma in a Taiwan population. Oral Oncol 2008; 44: 798-804.*
24. Peters WH, Lacko M, Te Morsche RH, Voogd AC, Oude Ophuis MB, Manni JJ. *COX-2 polymorphisms and the risk for head and neck cancer in white patients. Head Neck 2009; 31: 938-43.*
25. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. *Interleukin-10. Annu Rev Immunol 1993; 11: 165-90.*
26. Pratesi C, Bortolin MT, Bidoli E, Tedeschi R, Vaccher E, Dolcetti R, Guidoboni M, Franchin G, Barzan L, Zanussi S, Caruso S, De Paoli P. *Interleukin-10 and interleukin-18 promoter polymorphisms in an Italian cohort of patient with undifferentiated carcinoma of nasopharyngeal type. Cancer Immunol Immunother 2006; 55: 23-30.*

 DOI: 10.3346/jkms.2010.25.7.1024