Genetic Susceptibility to Lung Cancer in Koreans

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Lung cancer is frequently cited as an example of malignancy determined solely by exposure to environmental carcinogens such as cigarette smoking. However, only a fraction of smokers (10–20%) develop lung cancer, suggesting that there may be differences in individual susceptibility to lung cancer. The risk of lung cancer increases with family history, particularly the apparent effect of family history on female, non-smoker and young adult, also provides evidence supporting an inherited component for lung cancer (1).

The concept that people differ in their susceptibility to disease is not a new hypothesis. As early as the 5th century, Hippocrates mentioned “Some men have constitutions that are like wooded mountains running with springs, others like those with poor soil and little water, still others like land rich in pastures and marshes, and yet others like the bare, dry earth of the plain”. Today, we describe these observations as interindividual variation in disease risk manifested as gene-environment interactions, which embodies the concept that genetic factors modify the effects of environmental exposures (2).

The heritable effect of genes in cancer pathogenesis range from high-penetrance genes to low-penetrance genes. The high-penetrance genes are necessary and sufficient to cause disease, are rare in the populations, and are associated with strong familial aggregation and large relative risks, with onset of disease at an early age (e.g., BRCA1/breast cancer and RB/retinoblastoma). The low-penetrance genes are neither necessary nor sufficient for disease occurrence, and are associated with moderate familial aggregation (3). Individual variation in low-penetrance genes is usually because of genetic polymorphisms, which are changes in the nucleotide sequence that are present in at least 1% of the population (4).

Although lung cancer has been shown to occur, on occasion, in LiFraumeni families, associated with inherited p53 mutations, a high-penetrance gene for lung cancer has not yet been identified. Therefore, this succinct review will focus on polymorphisms of low-penetrance susceptibility genes involved in carcinogen metabolism, DNA repair and cell cycle control.

### METABOLIC ENZYME POLYMORPHISMS

Tobacco smoke is a complex mixture of more than 50 carcinogens, tumor initiators or promoters. However, the most important causative agents for lung cancer are polycyclic aromatic hydrocarbons (PAHs) and tobacco-specific N-nitrosamine (TSN) such as 4-(methylamino)-1-(3-pyridyl)-1-butanone (NNK). PAHs such as benzo[a]pyrene (BaP) preferentially induce squamous cell carcinoma, while TSNs exclusively induce adenocarcinoma.

The majority of carcinogenic chemicals does not produce their biological effects per se, but require metabolic activation before they can interact with cellular macromolecules. The xenobiotics-metabolizing machinery contains two main types of enzymes: the phase-I cytochromes P-450 (CYP) activating procarcinogens to genotoxic electrophilic metabolites, and phase-II conjugating enzymes such as glutathione S-transferase (GST) and N-acetyltransferase (NAT). Individual differences in susceptibility to carcinogens are thought to result from the balance between the formation of genotoxic intermediates and detoxification. Individuals who rapidly move through phase I metabolism and are deficient in phase II metabolism, resulting in the accumulation of genotoxic intermediates, are presumed to be at increased risk of lung cancer.

1) Cytochrome P450 (CYP, P450 family)

In mammals, more than 60-200 CYPs have been identified. The nomenclature system for CYPs includes the italicized capital symbol ‘CYP’ denoting human cytochrome P450, an Arabic number designating the family, a letter indicating the subfamily, and an Arabic numeral representing the individual gene within the subfamily.

The major human CYP forms in subfamilies CYP1 through CYP3, and has overlapping but distinct substrate specificities: CYP1A1 and CYP2C activate PAHs; and CYP2A3, CYP1A2, CYP2D6, and CYP2E1 activate N-nitrosamine.

(I) CYP1A1: The CYP1A1 gene is located on chromosome 15q22-24, and codes for aryl-hydrocarbon hydroxylase (AHH) involved in the metabolic activation of PAHs such as BaP.

Several polymorphisms in the CYP1A1 gene, including T6235C (3’ non-coding region, Mspl polymorphism), C4887A (Thr461Asn, exon 7), A4889G (Ile462Val, exon 7), and T5639C (intron 7) have been identified.

In the Japanese studies, CYP1A1 polymorphisms (Mspl and Ile462Val polymorphism) were associated with a 2–3 fold increased risk for lung cancer, especially for squamous cell carcinoma. This lung cancer susceptibility depended on cigarette dose, showing a high relative risk at a low level of cigarette smoking for individuals with susceptible genotypes (5–7). However, studies in Caucasians did not show clear associations between the CYP1A1 genotype and susceptibility to lung cancer (5–9). The racial or ethnic specific effects of CYP1A1 polymorphisms on lung cancer could be explained by

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a couple of reasons. A major possible explanation may be an ethnic difference of allele frequency of the polymorphism. The frequencies of MspI genotypes (m1/m1, m1/m2, m2/m2) are 49%, 40%, and 11%, respectively, in Japanese and are 78~84%, 16~20%, and 0.3~2%, respectively, in Caucasians. The frequency of Ile462Val polymorphism also shows large inter-ethnic differences (the frequency of Val allele: 20% in Japanese; 14% in Chinese; and 3.4% in Caucasians) (6,8). Therefore, studies in Caucasians with lower frequencies of variant alleles may be underpowered to detect risk differences. Another explanation for ethnic-specific effects may be related to differences in linkage or genetic association between alleles in different populations. The MspI polymorphism is closely linked in Japanese and less so in Caucasians with an Ile462Val polymorphism, suggesting that ethnic differences in genetic linkage disequilibrium are one possible mechanism for ethnic-specific effects on cancer susceptibility.

In Koreans, the frequencies of MspI genotypes (m1/m1, m1/m2 and m2/m2) are 36~40%, 46~49%, and 11~17%, respectively (5,6). My colleague and I reported that the m2/m2 genotype was associated with an increased risk for small cell lung cancer [odds ratio (OR)=3.38, 95% confidence interval (CI)=1.092-10.421] but not significantly associated with other histological types of lung cancer (5). We also evaluated the frequencies of Ile462Val polymorphism and an association between this polymorphism and lung cancer. The frequencies of Ile/Ile, Ile/Val and Val/Val genotypes were 60%, 38% and 2%, respectively (frequency of Val allele: 24%), similar to those reported in Koreans (6,8). The Val/Val genotype was not associated with a significantly increased risk for lung cancer (OR=2.76, 95% CI=0.50-19.95). Considering the high frequencies of variant alleles (m2 and Val alleles) in Koreans as in Japanese, it is highly probable that CYP1A1 polymorphisms contribute to genetic susceptibility for lung cancer in Koreans. Therefore, larger studies with more subjects are needed to clarify the association of CYP1A1 polymorphisms with lung cancer.

We also investigated the correlation of CYP1A1 Thr461Asn and T639C polymorphisms with lung cancer. The frequencies of these polymorphisms were very low as reported by Kim et al (8) and these polymorphisms had no significant association with lung cancer.

(2) CYP2D6: CYP2D6 metabolizes a wide range of nitrogen-containing drugs, including neuroleptics and beta-blockers. It also metabolizes the TSs such as NNK to mutagenic products. Individuals vary greatly in their CYP2D6 metabolic capacity, which is usually expressed as the metabolic ratio of debrisoquine metabolites to debrisoquine in urine. It was thought that poor metabolizers (PMs) might be less able to activate carcinogens in tobacco smoke, and thus be at reduced risk of lung cancer.

Recently, PCR-RFLP analysis made genotyping possible, and null allele (2D6D, gene deletion), poor metabolizing alleles (2D6A, A2637 deletion in exon 5; and 2D6B, G1934A at intron 3/exon 4 splice site), and a rare ultrarapid allele due to amplification of the CYP2D6 has been described. The frequencies of null and PM alleles (2D6D, 2D6A and 2D6B alleles) are significantly lower in Chinese (5.7%, 0% and 0.4%, respectively) than those of Caucasians (4.0%, 2.3% and 33.3%, respectively).

A recent meta-analysis concludes that individuals characterized as PMs, either by phenotype or genotype, are at reduced risk of lung cancer (OR=0.69, 95% CI=0.52~0.90). However, another meta-analysis reported no significant association between CYP2D6 polymorphisms and the risk for lung cancer (10). In Koreans, PMs are quite rare (11,12). Therefore, it is unlikely that CYP2D6 polymorphisms are an important genetic factor for lung cancer susceptibility in Koreans.

(3) CYP2E1: CYP2E1 is a major ethanol inducible P450 isoenzyme, and play an important role in the metabolic activation of various nitrosamines, including several potent tobacco-specific procarcinogens. In addition, it effectively reduces dioxygen to radical species, thus contributing to lipid peroxidation and oxidative stress.

The relationship between CYP2E1 Dral genotype (T7668A, intron 6) and lung cancer was first investigated in a Japanese study, where the individuals homozygous for a less frequent variant allele (C) were at reduced risk for lung cancer. Other studies in Caucasian populations have revealed a much lower frequency of variant C allele and show no significant relation between this polymorphism and lung cancer. A recent study performed in Hawaii showed that the variant allele was associated with a 5 fold decreased risk for lung cancer, with a 10 fold decreased risk for adenocarcinoma. These contradictory results are very intriguing and will need to be verified in studies with more statistical power from various ethnic populations. Two other RFLPs (G-1259C and C-1019T) have been described, revealed by either PstI or Rsal. These polymorphisms were not associated with lung cancer in Japanese and Brazilian studies.

In Korean, the frequencies of Dral genotypes (DD, DC and CC genotypes) are 63%, 33%, and 4%, respectively (13), which are similar to those in Japanese (59%, 30% and 11% respectively) and in Hawaiians (68%, 27% and 5%, respectively). My colleague and I investigated an association between the Dral polymorphism and lung cancer in Koreans. The frequencies of Dral genotypes (DD, DC and CC genotypes) among healthy controls were 59.1%, 35.4%, and 5.5%, respectively, and the individuals having CC genotypes were at a reduced risk for adenocarcinoma but not statistically significant. Lee et al. (14) reported that Rsal polymorphism was associated with the risk for lung cancer, especially in light smokers. However, we did not discover a statistically significant association of Rsal polymorphism with lung cancer. Since the frequency of CC genotype is low among healthy Koreans, further large investigations are warranted to clarify the association of these polymorphisms with lung cancer.

All CYP2E1 polymorphisms reported in the literature are located in noncoding regions of CYP2E1. Recently, two polymorphisms [G1168A (Arg76His, exon 2) and G10059A (Val389Ile, exon 8)] were identified in coding regions of CYP2E1. My colleague and I investigated the frequencies of these two polymorphisms in healthy Koreans. The frequencies of Arg76His and Val389Ile polymorphisms were 0.4% and 0%, respectively, suggesting that these polymorphisms do not have clinical significance.

2) Myeloperoxidase (MPO)

MPO, a heme-containing enzyme, which is present in the
lysosomes of neutrophils, plays an important role in bacterial and fungal killing by these cells. Exposure to a variety of pulmonary insults, including cigarette smoking, stimulates recruitment of neutrophils into lung tissue with local release of MPO. MPO activates carcinogens in tobacco smoking including BaP and aromatic amines and catalyzes the endogenous formation of carcinogenic free radicals.

The polymorphism (G-463A) in the promoter region of the MPO gene decreases gene expression by destroying a binding site for the general transcription factor SP1. Thus, individuals with the variant A allele may be provided with a protective effect due to decreased metabolic conversion of carcinogenic compounds in tobacco smoke. London et al (15) initially reported that subjects homozygous for the Variant A allele were at decreased risk of lung cancer: 70% for Caucasians (OR=0.30, 95% CI=0.10-0.93) and 39% for African-Americans (OR=0.61, 95% CI=0.26-1.41). Studies in German and Japanese also showed that the variant allele had a protective effect on lung cancer. My colleague and I found that individuals having A/A genotype were at reduced risk of lung cancer in Koreans (16).

3) Microsomal epoxide hydrolase (mEH)

Epoxide hydrolase (EH) is a phase II enzyme, which catalyzes the conjugation PAH epoxide. Although the products of hydrolysis are less reactive than the parent epoxide, some trans-dihydriodiol generated from PAHs are substrates for additional metabolic changes to highly toxic, mutagenic and carcinogenic polycyclic hydrocarbon diol epoxides. Thus, EH plays a dual role in detoxification and activation of carcinogens. Several polymorphisms have been described in the human mEH gene, and two of these (Try113His in exon 3 and His139Arg in exon 4) are associated with variations in enzyme activity. Substitution of the histidine at position 113 decreases mEH activity by approximately 40%, while a substitution of arginine at position 139 increases mEH activity by at least 25%. These polymorphisms could thus play a role in the etiology of lung cancer.

In an initial European Caucasian study, the slowest haplotype (His113/His139) was associated with an increased risk for lung cancer although statistically not significant. Benhamou et al. (17), in their study of squamous and small cell lung cancer, reported an association in the opposite direction: an increased risk for lung cancer (OR=2.66, 95% CI=1.33–5.33) with higher mEH activity. In a recent study, the very low mEH activity was associated with a decreased risk for lung cancer (OR=0.10, 95% CI=0.01–0.83) in African-Americans, but not in Caucasians. Differences in associations between ethnic subgroups or between study populations can result from linkage disequilibrium with additional allelic variants that modulate enzyme activity, and may be present in different frequencies in the different groups, or perhaps linkage disequilibrium with another gene that is causally related to lung cancer. Given the modest number of cases in each study, however, variability may be due to chance.

In Koreans, the frequencies of Try113His genotypes (Try/Try, Try/His and His/His) are 26%, 32% and 42%, respectively, which are different from those of Caucasians (45–55%, 31–49% and 6–15%, respectively) and those of African-Americans (68%, 31% and 1%, respectively). The frequencies of His139Arg genotypes (His/His, His/Arg and Arg/Arg) in Koreans are 72–75%, 22–26% and 1.5–2%, respectively, which are similar to those of Caucasians (62–72%, 26–35% and 2–4%, respectively) but different from those of African-Americans (45%, 45% and 10%, respectively). Combined with the above frequencies, it is likely that individuals with the fast haplotype are more common in Koreans than in Caucasians and African-Americans.

By the literature reported to date, mEH genotype was not associated with chronic obstructive pulmonary disease and lung cancer in Koreans.

4) Glutathione S-Transferase M1 (GSTM1)

Glutathione S-transferases (GSTs) are a group of phase II enzymes that detoxify diverse electrophilic, including carcinogens, chiefly by conjugating them with glutathione. In humans, there are four classes of cytosolic GST isoenzymes, namely alpha (GSTA), mu (GSTM), pi (GTP) and theta (GSTT). They have overlapping but different specific activities and affinities for electrophilic substrate: GSTM1, GSTM3 and GSTP1 preferentially detoxify PAHs metabolites, while GSTT1 is mainly involved in detoxification of ethylene oxide and butadiene. In human beings, the GSTM1 gene is polymorphic, and the phenotypic absence of enzyme activity is due to a homozygous inherited deletion of the gene, the null genotype. Individuals with GSTM1 null genotype are less able to detoxify metabolites of environmental carcinogens and therefore may be presumably at an increased risk of lung cancer.

A number of studies have been conducted to evaluate the potential role of GSTM1 null genotype as a risk factor for lung cancer. An earlier review of 12 case-control studies concluded that GSTM1 deficiency is a moderate risk factor for all histological subtypes of lung cancer with OR of 1.41 (95% CI=1.2–1.6). However, when these studies were stratified to race, an elevated OR was detected in Japanese population (OR=1.60, 95% CI=1.3–2.1), but not in Caucasians (OR=1.17, 95% CI=0.98–1.40). Moreover, the overall OR of lung cancer risk associated with GSTM1 deficiency in the studies determining GSTM1 status by genotyping was lower (OR=1.13, 95% CI=1.04–1.25) than that of studies based on phenotyping methods (OR=2.12, 95% CI=1.43–3.13). These results may suggest that the estimates of lung cancer risk associated with GSTM1 deficiency in the early studies determined GSTM1 status by phenotyping methods were exaggerated. Thus it seems reasonable that the role of GSTM1 deficiency as a lung cancer risk factor should be assessed in each ethnic population differently.

In Koreans, the frequency of GSTM1 null genotype is 50–55% (5,13,16,18), which is not significantly different from those in Caucasians (38–67%), Eastern Asians including Japanese and Chinese (33–63%). Studies in Koreans showed that GSTM1 null genotype was associated with an increased risk for lung cancer, especially squamous and small cell carcinoma (5,18).

DNA REPAIR GENE POLYMORPHISMS

DNA repair systems are fundamental to the maintenance of genomic integrity in the face of replication errors, environ-
mental insults and the cumulative effect of age, and their inactivation can dramatically increase individual susceptibility to cancer. Molecular cloning of DNA repair genes paved the way for possible application of polymorphisms as genetic markers for susceptibility to various cancers. In human, more than 70 genes are involved in the five major DNA repair pathways: direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair and double strand break repair. In this paper, I described the associations between the polymorphisms involved in BER and NER, and lung cancer.

1) BER gene polymorphism

BER is the main repair pathway for removing small base adducts produced by oxidation, methylation and radiation, and it occurs in multiple steps. The first step is the recognition and removal of the altered base by a DNA glycosylases catalyzing cleavage of the N-glycosyl bond between the modified base and the sugar moiety, leaving an abasic apurinic/apyrimidinic (AP) site in DNA. An AP endonuclease cleaves 5' to the AP site and a phosphodiesterase releases the remaining 5'-deoxyribose phosphate to leave a single strand break (SSB). Subsequently, the resulting gap is filled and sealed by the successive actions of a DNA polymerase and a DNA ligase.

A number of studies have been conducted to evaluate the potential roles of the polymorphisms of human 8-oxoG glycosylase (hOGG1) and X-ray repair cross-complementing group 1 (XRCC1) genes on the risk for lung cancer.

(1) Human 8-oxoguanine glycosylase 1: 8-hydroxyguanine (8-oxoG) is one of the major form of oxidative DNA damage produced by reactive oxygen species such as O$_2^-$ and H$_2$O$_2$. Since 8-oxoG preferentially pairs with adenine instead of cytosine during DNA replication, 8-oxoG causes GC $\rightarrow$ TA transversions, commonly seen in lung cancer where these transversions are very frequently found in the p53 gene. The hOGG1 gene encodes a DNA glycosylase/AP-lyase, catalyzing the excision of 8-oxoG, and expressed in seven major alternative splicing isoforms. Among these, type Ia has a nuclear localization signal and is involved in the repair of 8-oxoG.

Several polymorphisms in the hOGG1 gene have been identified, but the Ser326Cys polymorphism has been widely studied in relation to lung cancer. The frequency of Cys allele in Koreans is 51% (19), which is lower than in Chinese (61%) but higher than in Japanese (41–43%) and Caucasians (22–24%). Sugimura et al. (20) reported that individuals with the Cys/Cys type were at an increased risk for lung cancer in Japanese (OR=1.71, 95% CI=0.92-3.19), especially squamous cell carcinoma (OR= 3.01, 95% CI=1.33-6.83). However, no significant association was observed in Caucasians and in Koreans (19).

(2) X-ray repair cross-complementing group 1: XRCC1 gene encodes a multidomain protein that functions in repair of SSBs in DNA, through its interaction with poly (ADP-ribose) polymerase (PARP), DNA polymerase $\beta$ and DNA ligase III. The XRCC1 protein interacts with PARP through a unique central BRCT (BRCA1 C-terminus) domain specifically at amino acids 384–476. In BER, PARP is involved in the detection of DNA strand breaks and possibly in the recruitment of XRCC1, and catalyzes the transfer of ADP ribose from its substrate NAD to a number of nuclear receptors. Three coding polymorphisms at conserved sites have been identified in XRCC1 gene (Arg194Trp, Arg280His, and Arg399Gln). Most of studies have been focused on the Arg399Gln polymorphism since this polymorphism occurs in functionally significant PARP binding domain.

A few studies have investigated the potential role of XRCC1 codon 399 polymorphism on lung cancer risk, but the results were inconsistent. In a Polish study, there was no association of lung cancer with this polymorphism. Divine et al. (21) reported that the Gln/Gln genotype was associated with an increased risk of adenocarcinoma: the risk estimates for the risk genotype was much higher in non-Hispanic Whites than in Hispanics. In Koreans (22), the frequency of the Gln allele was 0.22, which was lower than that in Chinese and Taiwanese (0.26), and Caucasians (0.32–0.37). This polymorphism was associated with squamous cell carcinoma (Arg/Gln genotype: OR=1.45, 95% CI=0.84-2.50; and Gln/Gln genotype: OR= 3.26, 95% CI=1.17-9.15) but not with adenocarcinoma in Koreans. The reason for these discrepancies between studies is unclear. However, the different results in different populations may be due to genetic and environmental differences.

2) Polymorphisms of NER genes

NER targets mainly bulky, helix-distorting adducts such as BaP-guanine adducts, and occurs in multiple steps. In the first step, 15–18 polypeptides in six repair factors act in concert to excise DNA damage in the form of 24–32 nucleotide long oligomer. In the second step, gapped DNA is a substrate for replicative synthesis by a polymerase $\delta$ or $\varepsilon$ holozyme with the replication factors RPA (replication protein A), PCNA and RFC (replication factor C) and the sugar-phosphate backbone is sealed by a DNA ligase.

(1) Xeroderma pigmentosum group A (XPA): XPA gene is located on chromosome 9q22.3 and encodes a zinc-finger protein of M, 31,000 (273 amino acids). XPA protein is anticipated to verify NER lesions and to play a central role in positioning the repair machinery correctly around the injury through its interaction with RPA, TFIH (transcription factor IHI) and ERCC1 (excision repair cross-complementing group 1)-XPF. Even with the potential importance of XPA gene in carcinogenesis, none has investigated the role of polymorphisms of XPA gene in relation to cancer. My colleague and I recently reported that XPA polymorphism was associated with risk for lung cancer (23).

(2) XPC: XPC protein is complexed with the hHR23 (human homolog of the yeast NER factor Rad23) repair protein and plays a central role in global genome NER (GG-NER). The XPC-hHR23 complex functions as an early damage detector and a molecular matchmaker for recruitment of other components of the repair apparatus to the damaged site in GG-NER.

Three coding polymorphisms (Arg492His in exon 8, Val499Ala in exon 8 and Lys939Gln in exon 15) have been identified. Recently, poly (AT) insertion/deletion polymorphism in the intron 6 was reported. Shen et al. (24) reported that this intronic PAT polymorphism was associated with an increased risk for head and neck squamous cell carcinoma. My colleague and I investigated the association of these polymorphisms with lung cancer. In Koreans, Lys939Gln and PAT polymorphisms had no significant association with lung cancer, and Arg492His and
Val499Ala polymorphisms were not observed.

(3) XPD: XPD protein is an ATP-dependent 5'-3' helicase, a subunit of TFIIH that is essential for transcription and NER. A few studies reported that XPD codon 751 polymorphism was associated with DNA repair capacity and upper aerodigestive tract cancer risk. However, the codon 751 polymorphism was not significantly associated with lung cancer in Koreans (25).

CONCLUSIONS

I described the potential role of genetic variants of xenobiotics-metabolism and DNA repair on lung cancer risk. As described here, it would be premature to form definite views of relevance of these genetic variants to lung cancer risk due to ethnic differences in gene structure and allele distribution (e.g., rare alleles, gene amplifications and pseudogenes). Therefore, further studies involving larger cases are necessary to detect Korean-specific genetic susceptibility factors.

ACKNOWLEDGEMENTS

This study is supported in part by the KOSEF through the Biomolecular Engineering Center at Kyungpook National University.

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