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Genetic Basis for Susceptibility to Lung Cancer: Recent Progress and Future Directions

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Lung cancer is the leading cause of cancer death worldwide, and cigarette smoking is the major environmental factor for its development. To elucidate the genetic differences in the susceptibility to lung cancer among individuals, genetic factors involved in tobacco-induced lung cancers have been extensively investigated and a number of genetic polymorphisms have been identified to date as candidates. Most of the polymorphisms identified are of genes encoding proteins associated with the activity to metabolize tobacco smoke carcinogens and to suppress mutations induced by those carcinogens, and functional significances have been elucidated for some of these polymorphisms. However, the significance of these polymorphisms in the contribution to lung cancer development still remains unclear. Recently, several novel lung cancer susceptibility genes, including those on chromosomes 5p15.33, 6p21, and 15q24-25.1, have been identified by large-scale genome-wide association (GWA) studies. The 15q25 region contains three nicotine acetylcholine receptor subunit genes, and their polymorphisms have been also reported as being associated with nicotine dependence. The 5p15.33 region is associated with risks specifically for lung adenocarcinoma, the commonest histological type and weakly associated with smoking. This locus has been shown to be associated with risks for a wide variety of cancers, including lung adenocarcinoma. Associations of the 6q21 region have not been consistently replicated among studies. The 6q23-25 and 13q31.3 regions were also identified by recent GWA studies as being associated with risk for lung cancer, particularly in never-smokers. However, contributions of genetic differences on these five loci to the susceptibility to overall lung cancer seem to be small. There are several molecular pathways for the development of lung adenocarcinomas, and environmental factors for their development are still unclear,

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especially those in never-smokers. In addition, geographic differences as well as gender differences in lung cancer risk have been indicated. Furthermore, various genes identified by candidate gene association studies have not been reevaluated for their significance together with genes identified by GWA studies in the same population. Therefore, further studies will be necessary to assess the individual susceptibility to lung cancer based on the combination of polymorphisms in multiple genes, and to establish a novel way of evaluating the individual risk for lung cancer for its prevention.

I. INTRODUCTION: OVERVIEW OF STUDIES ON GENETIC AND ENVIRONMENTAL FACTORS INVOLVED IN LUNG CANCER SUSCEPTIBILITY

Lung cancer is the leading cause of cancer death worldwide (Sun *et al.*, 2007). Therefore, identification of genetic factors as well as environmental factors is very important in developing novel methods of lung cancer prevention. Since cigarette smoking is the major environmental risk factor for the development of lung cancer, genetic factors for tobacco-induced lung cancer have been extensively investigated by candidate gene association studies for many years. Genes involved in the metabolism of tobacco smoke carcinogens and genes involved in the repair of genetic alterations induced by those carcinogens have been the major targets of those investigations. In contrast, recent advances in molecular technology and knowledge of the distribution of genetic polymorphisms in the human genome have made it possible to identify genetic factors responsible for the development of common polygenic diseases, including lung cancer, by a genome-wide approach. Indeed, several loci containing candidate lung cancer susceptibility genes have been identified in recent years by genome-wide association (GWA) studies. One of the chromosomal loci identified was 15q24-25.1, and this region contained three genes encoding nicotinic acetylcholine receptor subunit genes. Since this locus has been also suggested to be associated with nicotine dependence, genetic susceptibility for nicotine addiction has come to be the major genetic factor for the development of lung cancer. However, epidemiologically, the incidence of lung cancers has been increasing in never-smokers, in women, and in Asian population, in recent years; therefore, lung cancers in smokers and those in never-smokers are now considered to be different diseases from each other. For this reason, identification of genetic factors as well as environmental factors for the development of lung cancers in never-smokers has also arisen as a major topic for prevention of these lung cancers. Accordingly, GWA studies for the identification of lung cancer susceptibility genes without association with smoking behavior are now also being extensively conducted. Therefore, when we discuss the genetic basis for susceptibility to lung cancer, three different critical points should be considered, as summarized in Fig. 1, in association with their functional significance in the

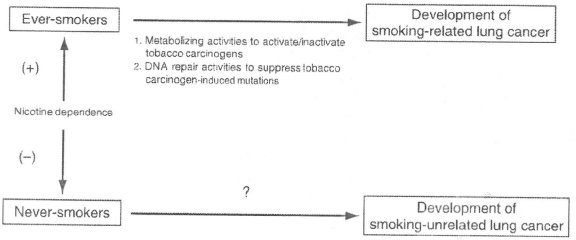

Fig. 1 Three different types of lung cancer susceptibility genes involved in the process of lung cancer development. Details are described in Section I.

susceptibility. The first point is interindividual differences in nicotine dependence, the second point is susceptibility to tobacco-induced (smoking-related) lung cancer, and the third point is susceptibility to lung cancer in never-smokers (smoking-unrelated). Nicotine dependence should be associated with the quantity of the intake of tobacco smoke carcinogens. In the development of smoking-related lung cancers, interindividual differences in metabolizing activities to activate/inactivate tobacco smoke carcinogens as well as DNA repair activities to suppress tobacco smoke carcinogen-induced mutations would play a major role. Since environmental factors for the development of lung cancer in never-smokers are largely unclear at present, GWA studies will be an effective approach to identify responsible genes for their development. In addition, we should accumulate the knowledge for the difference between lung cancers in smokers and those in never-smokers, from the viewpoint of molecular processes for their development. For this reason, in this review chapter, the differences between lung cancers in smokers and those in never-smokers are summarized first, recent progresses in lung cancer susceptibility gene studies are summarized second, and future directions of this field of science are discussed last.

II. DIFFERENCES IN THE PROCESS OF LUNG CANCER DEVELOPMENT BETWEEN SMOKERS AND NEVER-SMOKERS

Lung cancers are divided into the two major categories of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) from clinicopathological aspects (Sun *et al.*, 2007). NSCLCs are further divided into three

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major histological types, adenocarcinoma (ADC), squamous cell carcinoma (SQC), and large cell carcinoma (LCC). However, LCC is thought to be poorly or undifferentiated forms of and more heterogeneous than the other three types of lung cancer, and only limited information is available at present on genetic susceptibility to LCC. Therefore, in this review chapter, LCC is not specifically taken up as a subject for discussion. ADC, SQC, and SCLC are thought to be different in their origins (Govindan, 2010; Subramanian and Govindan, 2008; Travis *et al.*, 2004). Lung epithelial cells consist of monolayer columnar glandular epithelial cells (Fig. 2). Basal cells and neuroendocrine cells in the bronchi are thought to be precursors of SQC and SCLC, respectively. Clara cells in the bronchioles and/or type II pneumocytes in the alveoli are thought to be precursors of ADC. In mice, bronchioalveolar stem cells (BASC) have been identified as being a candidate precursor of ADC (Kim *et al.*, 2005); however, corresponding cells in the human have not yet been identified. Therefore, three major types of lung cancer are thought to be originated from different precursor epithelial cells in the lungs.

It is now widely accepted that cancer is attributed to accumulation of multiple genetic alterations in targeted precursor cells. Therefore, when we discuss genetic susceptibility to lung cancer, it is important to understand the differences and similarities in molecular pathways of cancer development among the three major types. Indeed, accumulated genetic alterations during their development are considerably different among them. Table I summarizes the accumulated genetic alterations in ADC, SQC, and SCLC (Govindan, 2010; Subramanian and Govindan, 2008; Sun *et al.*, 2007; Travis *et al.*, 2004). Ten genes identified to date with frequent genetic alterations in lung cancer cells are chosen as representatives. MYC, p53, PTEN, and PTPRD are genetically altered commonly among ADC, SQC,

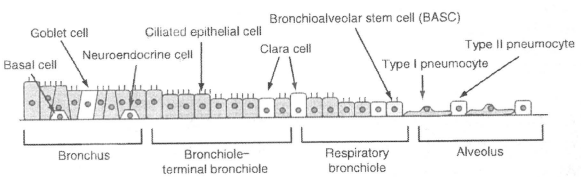

Fig. 2 Component epithelial cells in the pulmonary system. Precursor (progenitor) cells of small cell carcinoma, squamous cell carcinoma, and adenocarcinoma are thought to be neuroendocrine cells, basal cells, and Clara cells/type II pneumocytes, respectively. In mice, bronchioalveolar stem cells (BASCs) were identified as being precursor cells of adenocarcinoma; however, corresponding human cells have not identified to date.

Table I Oncogenes and Tumor Suppressor Genes Genetically Altered in Lung Cancer

Year[a]	Gene	Alteration	ADC	SQC	SCLC
1983	MYC[b]	Amp	+	+	++
1987	KRAS[b]	Mut	+[c]	–	–
1988	RB (RB1)[d]	Del, Mut	–	–	++[c]
1989	TP53[d]	Del, Mut	++	+++	+++
1994	p16 (CDKN2A)[d]	Del, Met, Mut	+[c]	+[c]	–
1998	PTEN[d]	Del, Met, Mut	+	+	++
2002	LKB1[d]	Del, Mut	++	–	–
2004	EGFR[b]	Small Del, Mut	++[c]	–	–
2005	PTPRD[d]	Del, Mut	+	+	+
2007	ALK[b]	Inv	+[c]	–	–

Abbreviations: ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC, small cell lung cancer; Amp, amplification; Mut, mutation; Del, deletion; Met, methylation; Inv, inversion.
[a]Year of genetic alterations identified in lung cancer cells.
[b]Oncogene.
[c]Occurrence of genetic alterations in a mutually exclusive manner
[d]Tumor suppressor gene.

and SCLC. RB and p16 are inactivated in a type-specific and mutually exclusive manner. Namely, RB is specifically inactivated in SCLC whereas p16 is specifically in ADC and SQC. KRAS, LKB1, EGFR, and ALK are specifically mutated in ADC. It is noted that KRAS, EGFR, and ALK are mutated in a mutually exclusive manner in ADC; therefore, in the light of accumulated genetic alterations, ADC can be further divided into at least four different types: KRAS-type, EGFR-type, ALK-type, and non-KRAS/EGFR/ALK-type (Govindan, 2010; Subramanian and Govindan, 2008; Travis *et al.*, 2004). EGFR-type is the major type of lung ADC in Asian people (30–50%) and the fraction of EGFR-type in Asian people is higher than that in American/European people (i.e., individuals of European decent; 10–20%), representing a geographic and/or ethnic difference in lung cancer development. Instead, the fraction of KRAS-type in American/European people (20–40%) is higher than that in Asian people (10%). The fraction of ALK-type is ~5% in both populations.

All three major types of lung cancer are associated with tobacco smoking; however, associations are much stronger in SQC and SCLC than ADC (Sobue *et al.*, 2002). Roughly speaking, more than 90% of patients with SQC or SCLC are smokers, whereas only ~50% of patients with ADC are smokers. Therefore, by considering the incidence of these three types of lung cancer, ~25% of lung cancer cases are not attributed to smoking. The proportion of patients with lung cancer in never-smokers (less than 100 cigarettes in their life time) is higher in Asian populations than American/European populations. In this subset of lung cancer, mostly classified into

ADC, environmental factors causing the accumulation of multiple genetic alterations have been poorly understood. In particular, EGFR-type and ALK-type are frequent in never-smokers, while KRAS-type and non-KRAS/EGFR/ALK-type are frequent in smokers.

LKB1 alterations preferentially accumulate in the KRAS-type, and both LKB1 and KRAS are genetically altered more frequently in American/European people than in Asian people. In contrast, EGFR mutations occur more frequently in Asian people than in American/European people, as described above. Therefore, even though both KRAS-type and EGFR-type are histologically classified into ADC, they are thought to be different diseases from each other (Govindan, 2010; Subramanian and Govindan, 2008; Travis *et al.*, 2004). From this point of view, it can be said that American/European populations are more susceptible to KRAS-type ADC, while Asian populations is more susceptible to EGFR-type ADC, due to the difference in either or both genetic and environmental factors.

III. CANDIDATE GENE ASSOCIATION STUDIES

Histological heterogeneity of lung cancer has been known for many years. However, lung cancers in never-smokers have not been classified into a different disease until recently. Therefore, in the last two decades, genetic susceptibility for tobacco-induced lung cancer has been extensively investigated by a candidate gene approach focusing on the metabolism of tobacco smoke carcinogens and the suppression of tobacco-induced genetic alterations. Lung cancer cells developed in smokers have been shown to have a unique mutation spectrum, with an excess of G:C to T:A transversions (Hollstein *et al.*, 1991; Le Calvez *et al.*, 2005). Therefore, associations of metabolic enzyme activities as well as DNA repair activities to induce or prevent G:C to T:A transversions have been a focus of genetic susceptibility studies in lung cancer (Govindan, 2010). Benzo[a]pyrene (BP) is a major polyaromatic hydrocarbon (PAH) in tobacco smoke, and benzopyrene-diol-epoxide (BPDE), a metabolite of BP (Alexandrov *et al.*, 2002; Rubin, 2001), forms a DNA adduct and induces G:C to T:A transversions at hot spot codons in the p53 gene in lung cancers of smokers (Le Calvez *et al.*, 2005). Cytochrome P450 (CYP)-related enzymes and glutathione-*S*-transferases (GSTs) are representative metabolic enzymes for tobacco smoke carcinogens because their polymorphisms have been extensively investigated in association with risk for lung cancer, particularly for SQC (Bartsch *et al.*, 2000). CYP1A1 bioactivates PAHs, such as BP, and a single nucleotide polymorphism (SNP) of Ile462Val in the CYP1A1 gene causes the difference in the enzymatic ability. The 462Val allele encodes a protein with a higher activity

to bioactivate PAHs than the 462Ile allele, and individuals carrying the 462Val allele have been shown to have higher risk to lung cancer than those carrying the 462Ile allele. In contrast, GSTs detoxify tobacco carcinogens such as PAH, and individuals lacking GSTM1 (null-type in an insertion/deletion polymorphism) have been shown to have an elevated risk for lung cancer. There have also been extensive works on the role of DNA repair genes as a determinant of inherited susceptibility to lung cancer (Schwartz *et al.*, 2007). For instance, in 1999, we first reported the possible contribution of OGG1 SNPs to lung SQC risk (Sugimura *et al.*, 1999). 8-oxo-deoxyguanosine (8-oxo-dG) is a major form of oxidative DNA damage induced by reactive free radicals and is highly mutagenic with frequent induction of G:C to T:A transversions both *in vitro* and *in vivo*. The OGG1 gene encodes an oxo-guanine DNA glycosylase that removes 8-oxo-dG from double-stranded DNA, thus preventing the occurrence of G:C to T:A transversions induced by 8-oxo-dG. The risk (326Cys) allele for the Ser326Cys SNP in the OGG1 gene encodes a DNA glycosylase with a weaker activity to repair 8-oxo-dG, in part produced by tobacco carcinogens, than the 326Ser allele (Kohno *et al.*, 1998; Yamane *et al.*, 2004). TP53 and MDM2 are also representative DNA repair genes associated with lung cancer risk (Bond and Levine, 2007; Imyanitov, 2009; Whibley *et al.*, 2009). The risk (72Pro) allele for the TP53-Arg72Pro SNP in the TP53 gene encodes a protein with a weaker apoptotic activity, thus allowing better survival of cells with DNA damages than the 72Arg allele. The risk (G) allele for a T/G SNP in the promoter region of the MDM2 gene (which is called MDM2 SNP309) allows a lower level of expression of MDM2 protein to suppress TP53 function than the T allele.

Table II summarizes lung cancer susceptibility genes identified to date by candidate gene association studies, and confirmed as being consistently associated with lung cancer risk by recent meta-analyses or pooled analyses of various studies (Dai *et al.*, 2009; Dong *et al.*, 2008; Kohno *et al.*, 2006; Li *et al.*, 2008; Wilkening *et al.*, 2007; Ye *et al.*, 2006). However, in most of these analyses, histological differences are not critically analyzed, probably because histological types were not available in some studies selected for meta-analyses. Therefore, the contribution of those polymorphisms to each histological type of lung cancers is not clear at present, although most polymorphisms are thought to be associated with the development of smoking-related lung cancer. In addition, results are different among several meta-analyses due to differences in the studies selected for meta-analyses. Such differences would be due to the quality of each study selected for the analysis. Therefore, to make uniform the quality among studies, several international consortiums have been established to date; thus, more reliable data will be available for various functional SNPs in the near future.

Table II Lung Cancer Susceptibility Genes Identified by Candidate Gene Association Studies (Meta-Analysis)

Gene	Gene product	Function	Polymorphism	SNP ID	Odd ratio	References
CYP1A1	Cytochrome P450	Phase I metabolism	Ile462Val	rs1048943	2.36	Dong *et al.* (2008)
mEH (EPHX1)	Epoxide hydrolase	Phase I metabolism	His113Tyr	rs1051740	0.70	Dong *et al.* (2008)
MPO	Myeloperoxidase	Phase I metabolism	G-463A	rs2333227	0.71	Dong *et al.* (2008)
GSTM1	Glutathione-*S*-transferase	Phase II metabolism	Presence/null	–	1.18	Ye *et al.* (2006)
GSTT1	Glutathione-*S*-transferase	Phase II metabolism	Presence/null	–	1.28	Dong *et al.* (2008)
XPA	Nucleotide excision repair protein	Mutation suppression	G-23A	rs1800975	0.73	Dong *et al.* (2008)
XPC	Nucleotide excision repair protein	Mutation suppression	Lys939Gln	rs2228001	1.30	Dong *et al.* (2008)
XPD	Nucleotide excision repair protein	Mutation suppression	Lys751Gln	rs1052559	1.30	Dong *et al.* (2008)
XRCC1	Base excision repair protein	Mutation suppression	Arg399Gln	rs25487	1.34	Dong *et al.* (2008)
OGG1	Base excision repair protein	Mutation suppression	Ser326Cys	rs1052133	1.32	Li *et al.* (2008)
OGG1 (ADC)					1.43	Kohno *et al.* (2006)
TP53	Transcription factor	Cell cycle/death regulation	Arg72Pro	rs1042522	1.20	Dai *et al.* (2009)
MDM2	Ubiquitine ligase	Cell cycle/death regulation	T309G	rs2279744	1.27	Wilkening *et al.* (2007)

IV. GENOME-WIDE ASSOCIATION STUDIES

Recent GWA studies have lead to the identification of a number of candidate lung cancer susceptibility genes (Table III). Three chromosomal loci, 15q24-25.1, 5p15.33, and 6p21, have been shown to be associated with lung cancer risk in Europeans and Americans (Amos *et al.*, 2008; Hung *et al.*, 2008; McKay *et al.*, 2008; Thorgeirsson *et al.*, 2008; Wang *et al.*, 2008). The chromosome 15q24-25.1 region contains the nicotinic acetylcholine receptor subunit genes, CHRNA3 and CHRNA5, and their products are expressed in pulmonary epithelial cells including neuroendocrine cells and bind to nicotine. Therefore, the association of this locus with lung cancer risk could be primarily mediated by nicotine dependence as described below. The 5p15.33 region contains the TERT (telomerase reverse transcriptase) gene and the CLPTM1L (cleft lip and palate transmembrane protein 1-like) gene. TERT is known to function in telomere replication and maintenance, and to promote epithelial cell proliferation. CLPTM1L was identified through screening for cisplatin (CDDP) resistance-related genes. Interestingly, this locus is associated with the risk for ADC but not for SQC or SCLC, suggesting the weak association of this locus with lung cancer risk in smokers (Landi *et al.*, 2009). Indeed, the 5p15.33 (TERT-CLPTM1L) genotypes were shown to be associated with lung ADC risk in never-smokers (Wang *et al.*, 2010). Associations of the 5p15.33 genotypes have been detected not only in lung cancer but also in various other types of cancers, including cancers of the brain, bladder, prostate, uterine cervix, and skin (Rafnar *et al.*, 2009; Stacey *et al.*, 2009). Therefore, it is likely that genotypes of this locus are associated with the development of a wide variety of cancers. Association with lung cancer risk of a SNP in the CHRNA3 gene at 15q24-25.1 was replicated in a Japanese population, although the frequency of the risk variant in the Japanese is much lower than that in Europeans and Americans (Kohno *et al.*, 2010). In a Chinese population, the association of SNPs in this locus with lung cancer risk was also replicated; however, risk variants seem to be different from Europeans and Americans (Wu *et al.*, 2009). Interestingly, in Asian populations, the associations of 15q24-25.1 SNPs with lung cancer risk were independent of smoking behavior. Associations with lung cancer risk of SNPs in the 5p15.33 region were validated in both Japanese and Chinese populations (Jin *et al.*, 2009; Shiraishi *et al.*, 2009), and the TERT gene was indicated to be a more likely target rather than the CLPTM1L gene.

The 6p21 region contains the BAT3 (HLA-B associated transcript 3) and MSH5 (mutS homolog 5) genes. BAT3 protein complexes with a histone acetyltransferase (HAT), p300, which acetylates p53 protein in response to DNA damage. MSH5 is a gene involved in DNA mismatch repair.

Table III Lung Cancer Susceptibility Genes Identified by Genome-Wide Association Studies

Chromosomal location	Gene	SNP/allele	Location	Minor allele frequency	Allele OR (95% CI, P)	Case/control	References
15q25.1	*CHRNA3*[a]	rs1051730	USA and UK	0.33	1.32 (1.23–1.39, 7.0×10^{-18})	2013/3062	Amos *et al.* (2008)
			Central Europe	0.33	1.30 (1.19–1.43, 5.4×10^{-9})	1922/2520	Hung *et al.* (2008)
			Iceland	0.35	1.31 (1.19–1.44, 1.5×10^{-8})	1024/32,244	Thorgeirsson *et al.* (2008)
			Europe, USA and Canada	0.35	1.31 (1.27–1.36, 1.9×10^{-51})	13,300/19,666	Landi *et al.* (2009)
			Japan	0.02	1.79 (1.19–2.78, 0.0095)	2343/1173	Shiraishi *et al.* (2009)
5p15.33	*TERT*[b]	rs2736100	Europe and Canada	0.49	1.19 (1.11–1.27, 2×10^{-6})	2971/3746	McKay *et al.* (2008)
			Europe, USA and Canada	0.50	1.12 (1.08–1.16, 1.6×10^{-10})	13,300/19,666	Landi *et al.* (2009)
			USA and Europe		1.15 (1.10–1.20, 1×10^{-10})	9162/11,812	Truong *et al.* (2010)
			Asia, USA and Canada		1.23 (1.12–1.35, 2×10^{-5})	1686/2101	
			China	0.42	1.16 (1.03–1.30)	1221/1344	Jin *et al.* (2009)
			Japan	0.38	1.38 (1.23–1.56, 6.3×10^{-8})	2343/1173	Kohno *et al.* (2010)
	CLPTM1L[c]	rs402710	Europe and Canada	0.35	0.82 (0.76–0.88, 3×10^{-7})	2971/3746	McKay *et al.* (2008)
			USA and Europe		1.14 (1.09–1.19, 5×10^{-8})	8860/9198	Truong *et al.* (2010)
			Asia, USA and Canada		1.15 (1.04–1.27, 0.007)	1680/2117	
			China	0.69	0.92 (0.81–1.03)	1221/1344	Jin *et al.* (2009)
			Japan	0.65	0.91 (0.81–1.03, 0.15)	2343/1173	Kohno *et al.* (2010)
		rs401681	USA, UK and central Europe	0.45	0.87 (0.84–0.92, 7.9×10^{-9})	5095/5200	Wang *et al.* (2008)
			Europe, USA and Canada	0.44	0.89 (0.86–0.92, 6.7×10^{-11})	13,300/19,666	Landi *et al.* (2009)
			Japan	0.33	0.88 (0.78–0.99, 0.044)	2343/1173	Kohno *et al.* (2010)
6p21.33	*BAT3*[d]–*MSH5*[e]	rs3117582	USA, UK and central Europe	0.10	1.24 (1.16–1.33, 5.0×10^{-10})	5095/5200	Wang *et al.* (2008)
			Europe, USA and Canada	0.10	1.22 (1.15–1.29, 4.8×10^{-12})	13,300/19,666	Landi *et al.* (2009)
			Japan	0	–	525/525	Kohno *et al.* (2010)
6p21.31	*HLA-DQA1*[f]	*03	Japan	0.36	1.36 (1.20–1.54, 5.3×10^{-7})	1656/1173	Kohno *et al.* (2010)

[a]Cholinergic receptor, nicotinic, alpha 3.
[b]Telomerase reverse transcriptase.
[c]Cleft lip and palate transmembrane protein 1-like protein.
[d]HLA-B associated transcript 3.
[e]mutS homolog 5.
[f]Major histocompatibility complex, class II, DQ alpha 1.

Therefore, both genes are attractive candidates for lung cancer susceptibility genes; however, a recent pooled analysis from the international lung cancer consortium did not replicate the association of these SNPs with lung cancer risk (Truong *et al.*, 2010). The significance of SNPs at 6p21 on lung cancer risk of Asians has not been fully investigated; however, our recent GWA study on the Japanese using 23,000 microsatellite markers for the screening indicated that the HLA-DQA1 gene, encoding a HLA (human leukocyte antigen)-class II protein, mapped at 6p21.31 is the most significant region at 6p21 (Jin *et al.*, 2009). DQA1*03 of the HLA-DQA1 gene was defined as a risk allele with odds ratio (OR) of 1.36 (95%CI = 1.21–1.54, $P = 5.3 \times 10^{-7}$) by analysis of 1656 ADC cases and 1173 controls. The HLA-DQA1 locus was mapped 1-Mb proximal to the BAT3–MSH5 locus. Therefore, we further examined a SNP in the BAT3–MSH5 locus, rs3117582, which showed a significant association in Europeans and Americans. It was monomorphic for the protective allele in the Japanese. We therefore examined seven SNPs in linkage disequilibrium (LD) with this SNP in Europeans (i.e., $D' = 1$ in the HapMap data); however, associations of these SNPs in the BAT3–MSH5 locus were weaker than genotypes of the HLA-DQA1 locus, and these SNPs comprised a distinct LD block from the locus containing the HLA-DQA1 gene. Therefore, it was concluded that the 6p21.31 region containing the HLA-DQA1 locus is a lung ADC susceptibility locus distinct from the BAT3–MSH5 locus at 6p21.33. In a recent meta-analysis, the associations of the BAT3–MSH5 locus were shown to vary among studies (Broderick *et al.*, 2009). Thus, it is possible that the BAT3–MSH5 locus and also the HLA-DQA1 locus could be affected by the difference in population structure since it is located near/in the locus for major histocompatibility complex, a highly polymorphic locus in the human genome. Therefore, further investigation of this region is warranted to conclude whether and how genotypes in this region are associated with lung cancer risk.

As described above, the incidence of lung cancer in never-smokers is increasing, and lung cancers in never-smokers are now considered to be a different disease from lung cancers in smokers. Therefore, there have been a few GWA studies for the identification of loci associated with lung cancer risk specifically of never-smokers. One of the regions identified is chromosome 6q containing the RGS17 gene, which encodes a member of the regulator of G protein signaling (RGS) family (Amos *et al.*, 2010). RGS17 was identified as a major candidate for familial lung cancer susceptibility gene at chromosome 6q23-25 (Liu *et al.*, 2010a; You *et al.*, 2009), and proliferation and tumorigenesis of human lung tumor cells in nude mice were inhibited by knockdown of RGS17 expression levels. Never-smoking individuals with a risk haplotype of this locus were shown to have a 4.7-fold higher risk than those without risk haplotypes. Another region identified by

a GWA study is chromosome 13q31.3 (Li *et al.*, 2010). This locus was identified by a four-stage association screening of lung cancers in never-smokers, and there was a strong correlation between genotypes of this locus and transcription levels of the GPC5 gene in normal lung tissues, with the high-risk allele linked with a lower level of transcription. Therefore, it was suggested that downregulation of GPC might contribute to the development of lung cancer in never-smokers. As described above, the TERT–CLPTM1L locus on chromosome 5p15.33 was also shown to be associated with lung cancer risk in never-smokers (Landi *et al.*, 2009; Wang *et al.*, 2010).

V. ASSESSMENT OF LUNG CANCER RISK IN EACH INDIVIDUAL BY COMBINED GENOTYPES (GENE–GENE INTERACTIONS)

For many years, gene–gene interaction has been investigated among candidate genes with functional polymorphisms. In particular, interactions among CYP-family genes and GST-family genes have been indicated by both molecular epidemiological studies and biological studies (Alexandrov *et al.*, 2002; Bartsch *et al.*, 2000; Schwartz *et al.*, 2007). Biologically, activities of CYP1A1 and GSTM1 are a critical determinant for the dose of carcinogenic BPDE and other DNA-reactive PAH; however, there has been no clear epidemiological evidence indicating the interaction between genotypes of CYP1A1 and GSTM1 on the risk for smoking-associated lung cancer risk, including SQC and SCLC. Since several lung cancer susceptibility genes have been identified by GWA studies, it is now very important to elucidate the interaction among their genotypes in the contribution to lung cancer risk. Then, we will be able to further develop a method to assess individual susceptibility to lung cancer based on the combined genotypes of several lung cancer susceptibility genes. However, up to the present, there have been only a few reports pursuing such an interaction. Here, we briefly summarize the results of three different studies investigating the effect of combined genotypes among genes identified by recent GWA studies on lung cancer risk.

In our recent study, we attempted to evaluate the combined effect among the HLA-DQA1, TERT, and CHRNA3 loci on lung ADC risk, because these three loci showed significant associations with lung ADC risk in the Japanese (Jin *et al.*, 2009). However, the frequency of the susceptible haplotype in the CHRNA3 gene in the Japanese (0.2) was much lower than in European and American populations (0.4); therefore, interaction of CHRNA3 genotypes with HLA-DQA1 and TERT genotypes was unclear in this analysis. However, when ORs were calculated according to the number of risk alleles for

the HLA-DQA1 and TERT genes, there was an increasing trend with increasing number of risk alleles (per risk-allele OR = 1.43, $P = 7.8 \times 10^{-16}$), reaching up to OD = 4.76 for carriers of all four risk alleles. Namely, individuals homozygous both for the DQA1*03 and minor TERT alleles were defined as high-risk individuals with an OR of 4.76 (95% CI = 2.53–9.47, $P = 4.2 \times 10^{-7}$). These two alleles independently (i.e., without a significant interaction) conferred the risk (P for interaction = 0.88). This result indicates that individuals highly susceptible to ADC can be defined by combined genotypes of HLA-DQA1 and TERT.

Recently, pooled analysis was performed for the replication of lung cancer susceptibility loci at chromosomes 15q24-25.1, 5p15.33, and 6p21 (Truong *et al.*, 2010). Associations between 15q24-25.1 variants and the risk for lung cancer were replicated in white ever-smokers; however, there were no such associations in never-smokers or in Asians. For the chromosome 5p15.33 region, statistically significant associations were confirmed in both whites and Asians. The 6p21 variants were not associated with the risk for lung cancer. Therefore, in this study, associations of the combined genotypes for the 15q24-25.1 locus (rs16969968) and the 5p15.33 locus (rs2736100 and rs402710) with the risk for lung cancer were further analyzed in whites. The OR of lung cancer risk for homozygotes of the three risk variants compared with individuals with no risk allele was 2.64 (95%CI = 1.86–3.74, $P=4 \times 10^{-8}$; per risk-allele OR = 1.15, $P = 1 \times 10^{-26}$). Liu *et al.* recently determined the cumulative association of four loci, 5p15.33, 6p21.33, 6q23-25, and 15q24-25.1, with familial lung cancer risk (Liu *et al.*, 2010a). The results indicate a stronger cumulative association of any combined genotype than any individual genotype with familial lung cancer. The risk for lung cancer was increased to 3- to 11-fold among those who had at least one copy of the risk allele at each locus in comparison with those who did not have any of the risk alleles.

The results of those three studies are consistent and indicate the cumulative effect of the SNPs in three chromosomal regions, 5p15.33, 6p21, and 15q24-25.1, on the genetic susceptibility to lung cancer, although interactions among those SNPs in lung cancer risk are unlikely.

VI. SMOKING-ASSOCIATED DIFFERENCES (GENE–ENVIRONMENT INTERACTIONS)

Cigarette smoking increases the risk for all three major histological types of lung cancers, although the risk is less for ADC than for SQC and SCLC (Govindan, 2010; Sobue *et al.*, 2002; Subramanian and Govindan, 2008;

Sun *et al.*, 2007; Travis *et al.*, 2004). The smoking habit is largely attributed to nicotine dependence, because nicotine is addictive. Therefore, although nicotine itself is not carcinogenic, it has been assumed that nicotine dependence is indirectly associated with lung cancer risk by primarily causing the smoking habit and consequently resulting in the increase of tobacco carcinogen intake (Hecht, 2004). Recent GWA studies have identified an association of a common variant in the chromosome 15q24-25.1 region with lung cancer susceptibility (Amos *et al.*, 2008; Hung *et al.*, 2008; Thorgeirsson *et al.*, 2008). The region of ~200 kb in size with high LD contains six genes, and three of them encode nicotine acetylcholine receptor subunits, CHRNA5/A3/B4. This locus has been identified as being associated with nicotine dependence and smoking quantity by several studies (Lips *et al.*, 2010; Liu *et al.*, 2010b; Spitz *et al.*, 2008; Thorgeirsson *et al.*, 2008). Furthermore, this locus has been also identified as being associated with risk for several smoking-related diseases, such as chronic obstructive pulmonary disease (COPD) (Pillai *et al.*, 2009) and peripheral arterial disease (PAD) (Thorgeirsson *et al.*, 2008). Associations of this locus with smoking quantity and several smoking-related diseases, including lung cancer, support that the CHRNA genotypes are at least, in part, indirectly associated with lung cancer risk through smoking behavior. In contrast, associations with lung cancer risk in never-smokers as well as associations with an earlier age of lung cancer onset indicate the direct association of genotypes with lung cancer risk in a smoking behavior-independent manner. Associations with lung cancer risk after adjusting smoking habit also support the direct effect of genotypes on lung cancer risk. For this reason, associations of the CHRNA3/A5/B4 genotypes with lung cancer risk have been extensively and carefully investigated together with those with smoking behavior and nicotine dependence. However, several inconsistent results have been reported to date; thus, further studies are warranted.

Another example of smoking-associated differences is CYP family–GST family gene polymorphisms associated with smoking-related lung cancers, as described. However, to our knowledge, modifications by smoking behavior and/or smoking quantity of the associations between those genotypes and lung cancer risk have not yet been critically analyzed to date. Importantly, a recent study further indicated the association of other CHRNA genes, CHRNB3 and CHRNA6, on chromosome 8p11, with smoking and nicotine dependence as well as lung cancer risk (Thorgeirsson *et al.*, 2010). Interestingly, in their study, the association was also observed between the chromosome 19q13 region and smoking behavior as well as lung cancer risk. The 19q13 region contains the CYP2A6 gene, whose products have an enzyme activity to oxidize nicotine and to activate procarcinogenic nitrosamines.

VII. NECESSITY OF FURTHER ASSOCIATION STUDIES

To obtain more conclusive information on the genetic basis for susceptibility to lung cancer, we will have to analyze all the polymorphic sequences in the human genome for association with susceptibility. Various SNP array platforms have been developed to date, and the numbers of SNPs analyzable in one platform have been increasing year by year. In 2010, over a million SNPs can be analyzed by a single SNP array. However, it has been assumed that there are at least 10 million SNPs with a minor allele frequency (MAF) $>1\%$ and 5 million SNPs with a MAF $>10\%$ (Chung *et al.*, 2010; Frazer *et al.*, 2009). Therefore, although recent GWA studies have lead to the identification of several lung cancer susceptibility genes, it is still possible that there are several additional SNPs involved in the susceptibility in the human genome. In particular, several functional polymorphisms which have been identified by candidate gene association studies to date are not mounted on major SNP array platforms used in previous GWA studies, such as Affymetrix 500 K/1 M and Illumina HumanHap 300/550. Therefore, at present, it is not possible to obtain association data for those functional polymorphisms together with those for SNPs identified by GWA studies using SNP arrays. For this reason, we recently performed an association study of lung SQC for genes identified by GWA studies (CHRNA3, TERT, and HLA-DQA1) and genes identified by candidate gene association studies (TP53, MDM2, OGG1, CYP1A1, and GSTM1), because associations of these candidate gene polymorphisms were not investigated in recent GWA studies due to the lack of probes to discriminate these polymorphisms in the platforms used for GWA studies (Kohno *et al.*, in press). Genotypes for the TP53 and OGG1 genes showed significant associations with SQC risk in addition to those for the CHRNA3 and HLA-DQA1 genes to similar extents. Therefore, it will be necessary to reevaluate the significance of polymorphisms identified only by candidate gene association studies in several populations together with SNPs identified by GWA studies. In addition, it has been assumed that rare variants with frequencies less than 1% would play much more important role than common SNPs with a MAF $>10\%$ for the susceptibility to various diseases (Ioannidis *et al.*, 2010; Knerr *et al.*, 2010; McClellan and King, 2010). Therefore, a further technological advancement is absolutely required for the assessment of the role of rare variants in cancer susceptibility. Genetic polymorphisms include not only SNPs but also structural variations and copy number variations (CNVs) (Feero *et al.*, 2010; Frazer *et al.*, 2009). However, structural variations and CNVs are not yet easily analyzable at the genome-wide level. A CNV at 1q21.1 was recently shown to be associated with neuroblastoma susceptibility (Diskin *et al.*, 2009); therefore, development of a novel and easy

analytical method for CNVs throughout the human genome will be also necessary to finally identify all types of genetic polymorphisms associated with lung cancer risk.

Allele frequencies of several lung cancer susceptibility genes are different among different ethnic and geographic groups. Therefore, contribution of each susceptibility gene to lung cancer risk, represented by OR, is also considerably different among different ethnic/geographic groups. Figure 3 shows the differences in the frequencies of risk alleles for representative lung cancer susceptibility genes among Japanese, Chinese, Europeans, and Africans. Risk alleles for the CYP1A1 and OGG1 genes are more frequent in Asians than Europeans and Africans. In contrast, the risk allele for the CHRNA5 gene is more frequent in Europeans than Asians. Accordingly, comparative studies of polymorphisms with different allele frequencies and ORs among different ethnic/geographic groups will enable us to clarify the differences in lung cancer susceptibility

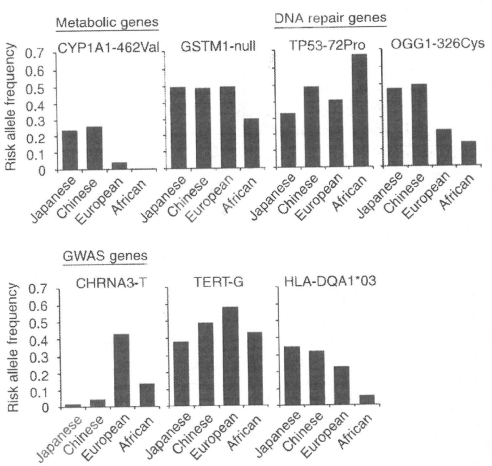

Fig. 3 Frequencies of risk alleles for lung cancer among different ethnic groups. As representatives, allele frequencies of seven genes in Japanese, Chinese, Europeans, and Africans are shown. Allele frequencies determined by the HapMap project, by the International Histocompatibility Working Group projects, by Ye *et al.* (2006), and by us (Kohno *et al.*, 2010; Shiraishi *et al.*, 2009) are combined in each column.

among different populations. For instance, lung ADCs with EGFR mutations in female nonsmokers are more common in Asian than in Americans and Europeans. However, it is still unknown whether or not such a difference is due to the difference in the distribution of risk alleles of some lung cancer susceptibility genes among these populations.

VIII. FUTURE DIRECTIONS

Recent GWA studies have identified three lung cancer susceptibility gene loci at chromosomes 15q24-25.1, 5q15.33, and 6p21. The 15q24-25.1 locus is associated not only with lung cancer but also with smoking behavior and other smoking-related diseases. Associations of the 15q24-25.1 genotypes with lung cancer risk in never-smokers and with lung ADC risk have been inconsistently observed among studies. In addition, the frequency of the risk allele is markedly different among ethnic groups. Therefore, further genetic studies as well as biological studies will be necessary to conclude whether the 15q24-25.1 genotypes play a direct or indirect role in the development of lung cancer, and how commonly/differentially the 15q24-25.1 genotypes contribute to lung cancer risk among different ethnic groups. The 5p15.33 locus is associated with risks not only for lung cancer but also for a variety of cancers, and the risk allele is prevalent among different ethnic groups. Therefore, this locus is likely to be associated with risks in general for a wide variety of cancers, irrespective of ethnic groups. Association of the 6p21 locus, containing the BAT3, MSH5, and HLA-DQA1 genes, with lung cancer has not yet been well reproduced by other genome-wide scale association studies. Therefore, further studies are necessary to obtain more convincing information for this locus in the association with lung cancer risk. Reevaluation of functional polymorphisms identified by candidate gene association studies will also be important for the assessment of individual risk for lung cancer (Wilkening *et al.*, 2009).

Lung cancers in never-smokers have been considered to be a different disease from those in ever-smokers. Associations have been observed between the 5p15.33, 6q23-25, and 13q31.3 genotypes and lung cancer risks in never-smokers. However, lung cancers in never-smokers are more common in women than in men, and also more common in Asian populations than in American and European populations (Govindan, 2010; Reid *et al.*, 2008; Subramanian and Govindan, 2008; Sun *et al.*, 2007). Therefore, it will be very important to elucidate interactions among genotypes, gender, and ethnicity/geography on lung cancer risk in never-smokers. The most frequent type of lung cancer in never-smokers is ADC; however, lung ADC is now considered to be a heterogeneous disease with respect to accumulated genetic alterations in cancer cells. EGFR-types are more frequent in Asian

populations, while KRAS-types are more frequent in American and European populations. However, it is still unknown whether such a difference is due to genetic differences or environmental differences. Identification of lung ADC susceptibility genes in never-smokers will facilitate the identification of environmental factors by subsequent functional analyses of identified genes. The elucidation of associations among such genetic factors, environmental factors other than smoking, and acquired genetic alterations in cancer cells will help us understand the molecular mechanisms underlying lung carcinogenesis in never-smokers and develop methods of its prevention.

Lastly, we point out here that none of polymorphisms have been identified yet to specifically define the risk for tobacco-induced lung cancer, such as SCC and SCLC. The 15q24-25.1 region containing three CHRNA genes is associated with smoking behavior as well as lung cancer susceptibility; however, it is still unclear whether this locus is associated with lung cancer risk among heavy smokers or not. Since only one in 10 smokers is estimated to develop lung cancer (Reid *et al.*, 2008), individual risks for lung cancer by smoking would be different due to genotype differences. Polymorphisms in genes for metabolism of tobacco smoke carcinogens and those for repair of carcinogen-induced genetic alterations have been considered as being candidates for many years. However, their significance is still unclear at present. Therefore, association studies of those genotypes with lung cancer risk by considering the smoking quantity, such as the number of cigarettes smoked per day (CPD), will be also important in assessing the individual risk for lung cancer in smokers. For this reason, the recently identified CYP2A6 gene locus will be another candidate to define lung cancer susceptibility in smokers (Thorgeirsson *et al.*, 2010).

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