CHAPTER IV

DISCUSSION

Lung cancer is the leading cause of cancer-related death and thus a major health problem (Parkin, 2005). Although most lung cancers are the consequence of smoking, a substantial fraction of molecular-epidemiological studies point to high-prevalence, low-penetrance genetic polymorphisms as modifiers of environmental lung cancer risk. Identifying such susceptibility polymorphisms may lead to the development of tests that allow a more focused follow-up of a high-risk group.

In Northern, Thailand the incidence of lung cancer has been the commonest cancer in both sexes (Sriplung, 2005, Vatanasapt, 2002). In this study, polymorphism profile of ten selected genes, previously described to show a significant impact on lung cancer risk (Geisler, 2001, Hung, 2005, Kiyohara, 2002, Kiyohara, 2005, Sugimura, 1999, Uematsu, 1991, Wang, 2003, Wang, 1999) was examined in Northern Thai population. The genotyping of CYP1A1(MspI), CYP1A1(Ilu462Val), CYP2E1(PstI), CYP2E1(DraI), MPO(AciI) and MMP-1(AluI) was carried out using PCR-RFLP, while genotyping of GSTM1 and GSTT1 was done by multiplex PCR. The missense mutation variant of hOGG1(Ser326Cys) and p53(Arg72Pro) was identified by di-allele-specific amplification with artificially modified primers (diASA-AMP) previously described by Liang (Liang, 2005). The genotype distribution of most of the investigated genes in healthy control was concordance with the Hardy-Weiberg equilibrium, except MPO(AciI) and MMP-1(AluI). The variant frequency of most of the investigated genotypes among the healthy controls in our

study was similar to those previously described in Caucasian, Asian and Thai population (as shown in Table 4.1). However, the frequency of GSTT1 null genotype and CYP1A1(MspI) variant genotype was slightly higher than previously reported in Caucasians (Ada, 2004, Dialyna, 2003).

Although a number of articles have been reported an association of the specific CYP450 isozymes, especially CYP1A1(MspI), CYP1A1(Ilu462Val) and CYP2E1(DraI), CYP2E1(PstI), as a genetic risk modulator of the lung cancer (Hayashi, 1992, Kawajiri, 1990, Uematsu, 1991, Uematsu, 1992), non of any CYP450 isoenzyme polymorphism, neither in isolation or in combination with others, showed a statistically significant impact on ORs (both crude and adjusted) of lung cancer risk in our study. This may partly due to the fact that a small group of samples were investigated, nevertheless, this lack of association between CYP450 polymorphism and lung cancer risk has also been reported by others (Kiyohara, 2003, London, 2000).

The effect of genetic polymorphism on lung cancer risk was further investigated by pairing different polymorphism together according to their tendency of increasing or decreasing the risk to see whether there is any enhanced effect between any two variant combinations. It was found that the null genotype of GSTM in combination with at least one variant allele of p53(Arg72Pro) or MMP-1(AluI) and at least one variant allele of hOGG1(Ser326Cys) in combination with at least one variant allele of p53(Arg72Pro) or MMP-1(AluI) or MPO(AciI) showed a significant effect of increasing lung cancer risk indicating their enhancing effect on each other. The association of similar combination genotypes with lung cancer risk has been reported previously (Dialyna, 2003). For MPO as the variant allele is related to low

metabolic activation activity, subjects with at least one variant allele was associated with decreased risk of lung cancer in a number of studies with Caucasian populations (Dally, 2002, Feyler, 2002, Le Marchand, 2000, Schabath, 2002). We found that MPO G-463A conferred no protective effect against lung cancer. Although it has been reported that A allele was more frequent (1.6 times) in Caucasians than in Asians (Kiyohara, 2005), the lack of protective effect of at least one variant allele of MPO G-463A has been report by the studies other than us both in Asian and Caucasian (Larsen, 2006, Yoon, 2008) population. Then again, the G/A and A/A genotypes, in concurrence with our study, was found to be associated with the increased risk of lung cancer in Caucasian older men (Misra, 2001). Therefore, the frequency is probably not the reason for all these contradictory reports about of MPO G-463A polymorphism and lung cancer risk. We believed that the varying in etiological factors of lung cancer in these different studied populations may (or partly) be the reason for these opposing results. It is possible that while variant genotype of MPO G-463A decreases the risk of cigarette smoke (Dally, 2002) it may enhance the risk of some other unknown lung carcinogens and/or mutagens. This is plausible given the dual role of MPO as an antimicrobial agent and as a regulator of inflammation; an independent association might be difficult to tease apart without knowing the requisite exposure. We also found that variant CYP2E1(PstI) polymorphism in combination with GSTT1 null genotype showed tendency to decrease lung cancer risk which has been previously reported by others (Le Marchand, 1998, Quinones, 2001, Sgambato, 2002, Wang, 2003); however, it was not statistically significant in our study.

Table 4.1 Frequencies of gene polymorphisms in different ethnic groups

Polymorphisms	Ethnic groups	No of subjects	Frequency of variant allele	References
CYP1A1 exon7 (Ilu462Val)	Caucasian	4,790	0.05	(Garte, 2001)
	Asian	1,132	0.23	(Garte, 2001)
	Japanese	1,25	0.22	(Murata, 2001)
	Korean	220	0.21	(Yang, 2007)
	Thai	287	0.30	(Pisani, 2006)
	This study	**80**	**0.32**	
CYP1A1 (MspI)	Caucasian	4,453	0.09	(Garte, 2001)
	Asian	638	0.36	(Garte, 2001)
	Japanese	146	0.43	(Wang, 2003)
	Korean	181	0.37	(Kim, 2000)
	Thai	286	0.30	(Pisani, 2006)
	This study	**82**	**0.52**	
CYP2E1 (PstI)	Caucasian	1,454	0.04	(Garte, 2001)
	Asian	719	0.23	(Garte, 2001)
	Taiwanese	446	0.24	(Wang, 1999)
	Japanese	181	0.26	(Wang, 2003)
	Korean	145	0.19	(Park, 2003)
	Thai	99	0.15	(Kongruttanachok, 2001)
	This study	**81**	**0.13**	
CYP2E1 (DraI)	Caucasian	1,360	0.07	(Garte, 2001)
	Asian	286	0.3	(Garte, 2001)
	Taiwanese	231	0.28	(Wang, 1999)
	Korean	138	0.22	(Park, 2003)
	This study	**81**	**0.25**	
GSTM1 (null)	Caucasian	10,514	0.53 (0.42-0.60)	(Garte, 2001)
	Asian	1,511	0.53 (0.42-0.54)	(Garte, 2001)
	Japanese	119	0.50	(Wang, 2003)
	Korean	346	0.52	(Yang, 2007)
	Thai	289	0.64	(Pisani, 2006)
	This study	**81**	0.58	
GSTT1 (null)	Caucasian	5,577	0.20 (0.13-0.26)	(Garte, 2001)
	Asian	575	0.47 0.35-0.52)	(Garte, 2001)
	Japanese	119	0.45	(Wang, 2003)
	Korean	345	0.48	(Yang, 2007)
	This study	**74**	**0.48**	
MPO (AciI)	Caucasian	5,107	0.23 (0.22-0.25)	(Kiyohara, 2005)
	Asian	1,660	0.14 (0.11-0.18)	(Kiyohara, 2005)
	Korean	432	0.09	(Park, 2006)
	This study	**81**	**0.11**	
hOGG1 (Ser326Cys)	Caucasian	5,451	0.21 (0.13-0.29)	(Hung, 2005)
	Asian	2,702	0.51 (0.33-0.69)	(Hung, 2005)
	Chinese	227	0.61	(Liang, 2005)
	This study	**75**	**0.58**	
p53 (Arg72Pro)	Caucasian	856	0.28 (0.21-0.37)	(Irarrazabal, 2003, Szymanowska, 2006, To-Figueras, 1996)
	Korean	181	0.33	(Kim, 2000)
	Thai	353	0.49 (0.43-0.52)	(Singto, 2004, Thaeomor, 2005, Tiwawech, 2003)
	This study	**79**	**0.6**	
MMP1 (AluI)	Caucasian	2,201	0.50 (0.48-0.54)	(Su, 2005, Wenham, 2003, Zhu, 2001)
	Japanese	150	0.62	(Kanamori, 1999)
	Chinese	350	0.74	(Fang, 2005)
	This study	**82**	**0.75**	

When the ORs of lung cancer risk in relation to the genetic polymorphism was further examined with stratification according to gender, it was found that heterozygous and homozygous variants of hOGG1(Ser326Cys) significantly increased the risk of lung cancer development among females and its impact was even more dominant in combination with at least one variant allele of p53(Arg72Pro) or MPO(AciI). Interestingly, the impact the null genotype of GSTM1 in combination with at least one variant allele of p53(Arg72Pro) or MMP-1(AluI) or hOGG1(Ser326Cys) on increasing lung cancer risk was also very influential in female, but only in a sub-group of non-smoking female.

Lung cancer incidence among Northern Thai women is one of the highest in Asia (Lam, 2005). A local cigar called “Khiyo,” which is made from shredded tobacco leaves and other flavorings such as tamarind shell and Koy-tree bark, wrapped with dry young banana leaves, has been suspected to generate the high incidence of lung cancer, since Khiyo contains a very high content of tar (28.5–200.8 mg per Khiyo) and has a high mutagenicity (Mitacek, 1991). Although tobacco smoking is believed to be the main causative factor for the incidence of lung cancer in this area (Simarak, 1977), it can not entirely explain the very high incidence of female lung cancer in Northern Thailand. Therefore, other risk factors such chronic benign respiratory diseases due to the fungus *Microsporum canis* ((Nakachi, 1999, Suttajit, 1994) or exposure to indoor radon radiation (Wiwatanadate, 2001) or mutagenic environmental air (Vinitketkumnuen, 2002) may also play the role.

The impact of multi-loci combination of GSTM1, MPO(AciI), hOGG1(Ser326Cys), p53(Arg72Pro), MMP-1(AluI) on lung cancer risk was further investigated by using a group of individual who carrying 2 or more wild-type

genotype of these 5 genes (positive genotype for GSTM1, homozygous wild-type for all the other genes) as a reference group. It was found that odd ratio of lung cancer risk for an individual who carrying 5 variants was higher than those carrying 4 variants and 4 variants was higher than those carrying 3 variants, respectively. Interestingly, when the OR was calculated with stratification of gender, the same, but more potent, pattern was observed among females but not among males.

Northern Thai women and Chinese women have a high rate of lung cancer despite a low prevalence of smoking, and studies in these populations have provided useful information on risk factors other than smoking. Environmental tobacco smoke, cooking oil vapors, indoor smoky coal burning, and infection with tuberculosis and human papillomavirus 16/18 are risk factors in different Chinese female populations (Gao, 1987, Ko, 2000). GSTM1, MPO(AciI) and hOGG1(Ser326Cys) are genes known to be involved in oxidative stress pathway. Oxidative stress can be initiated by a number of factors known to cause lung cancer, for example, cigarette smoking (van der Vaart, 2004), air pollution (Menzel, 1994), radon radiation (Bonner, 2006), *etc*. In fact, GSTM1 null genotype has been reported to increase the risk of lung cancer caused by radon irradiation (Bonner, 2006). Although MMP is not directly linked to oxidative stress, it has been reported that these enzymes are secreted by inflammatory cell in response to lung irritation (Imai, 2001) that can be caused by cigarette smoking, air pollution, radon radiation.

It is well recognized that p53 is referred to as “the guardian of the genome" since its response to genetic insult can trigger apoptosis or cell cycle arrest and checkpoint failure can give rise to genetic instability and tumorigenesis. One of the most important stimulation that leads to p53 activation is oxidative stress. Recent

studies have revealed the role of p53 in regulating reactive oxygen species (ROS) generation and ROS can act as both upstream signal that trigger p53 activation and down stream factor that mediate apoptosis (Liu, 2008). It has been suggested that *Pro72* encoded p53 protein is less effective in inducing apoptosis and suppressing cellular transformation than *Arg72* encoded p53 protein; therefore, this might explain the higher risk of an individual with Pro72 p53 to develop cancer compared to those with *Arg72* encoded p53 protein when exposing to the same oxidative DNA damage stimulation.

As there is low prevalence of smoker in females in comparison to males, the common risk factor capable of inducing oxidative stress among women who live in Northern region of Thailand could be either radon irradiation or cooking oil vapor. Although, in the past women are likely to be exposed to radon and cooking oil vapor as they spend most of their time in the house and do cooking, with modern life-style men and women are equally out-going and thus spend less time in the kitchen. The geographic location of Northern region span over mountain area and likely to contain Uranium underground that can generate radon radiation, however, there has been only one report in Chiang Mai on radon irradiation. Other provinces may or may not share the same problem, which needs to be further investigated. Another possible risk factor of lung cancer in Northern Thailand with the capability of inducing oxidative stress is air pollution (Moller, 2008) with the main source being from crop burning and traffic jam. Although, air pollution from traffic in northern Thailand is not as bad as Bangkok, with the geographic location that surrounding by mountains may some how empower their effects on human body. This may not provide the reason why women are more affected than men, other genetic background difference between male and

female, for example genes encoding sex steroid hormones as has been reported previously (Lam, 2005, Pauk, 2005), may also play the role, and this await further investigation.