CHAPTER V
DISCUSSION

Genetic polymorphism of NQO1 in Thai population

The genetic variation has important implications for more thorough understand of population history, evolution, and other applications. Several studies of genetic variation in phase II xenobiotic metabolizing enzyme genes have indicated a number of polymorphisms that modify enzyme activities (Gaedigk et al., 1998; Kelsey et al., 1997; Misra et al., 1998). Since NQO1 is an important enzyme in the metabolism of xenobiotics, variation in NQO1 enzyme activity may be an important factor for susceptibility to chemically-induced cytotoxicity and to various forms of cancer (Lyn-Cook et al., 2006; Mitrou et al., 2007; Moore et al., 2004; Park et al., 2003; Saldivar et al., 2005). Moreover, NQO1 enzyme polymorphisms across human populations are markedly different. A number of studies showed the role of NQO1 genetic polymorphisms in the susceptibility to human cancers or other diseases. However, there is only few reports demonstrated distribution of NQO1 genetic polymorphisms in Southeast Asian populations, especially in Thai population. This was the first report of genetic polymorphisms of *NQO1* gene in a Northeastern Thai population.

The NQO1 catalyzes the two-electron reduction of quinines to hydroquinones. In doing so, NQO1 protects the cell against cytotoxicity by reducing the concentration of free quinone available for single-electron reduction (Ross et al., 2000). This pathway is thought to be the major mechanism responsible for the toxicity of quinones, including those arising from benzo[a]pyrene, one of the most potent polycyclic aromatic hydrocarbons (Gelboin, 1980; Maltoni et al., 1983). The null enzyme activity (*NQO1*2*) allele of this enzyme is responsible by a single nucleotide polymorphism in the *NQO1* gene (NQO1 C609T) (Ross et al., 2000). This mutation has been associated with a decreased enzymatic activity, and increased risk of chemically-induced cytoxicity and susceptibility to various forms of cancer. Of particular interest is the recent finding that NQO1 has been found to play an important

role in regulating function of tumor suppressor (p53), by inhibiting its degradation (Asher and Shaul, 2006). This suggests that the lack of NQO1 activity can explain why individuals with a polymorphic inactive NQO1 are more susceptible to tumor development (Gong et al., 2007).

Currently, a number of genetic polymorphism of NQO1 has been identified and their frequencies vary among ethnic groups. The NQO1 wild type genotype was approximately twice more common among African-Americans than among Mexican-Americans ($p<0.001$) (Wiencke et al., 1997). Chen et al. (1999), found a notable difference in the frequency of the *NQO1*2* allele among Japanese (38%), Caucasians (20%), and Hawaiians (22%). Variation in allele frequency between difference ethnic groups has also been observed for other polymorphisms. Although the origin and consequence of the ethnic differences are not clear, it may help explain the inconsistent findings between different association studies.

In this work, the pattern of frequency distribution of *NQO1 *2* allele in Thais population has been found to be resembled to those East Asian (Oriental) (Choi et al., 2003; Hori et al., 2003; Zhang et al., 2003). This is distinct from those observed in Caucasian and African-Americans (Saldivar et al., 2005). Thus, this result also supported ethnic differences in the distribution of *NQO1* genotypes across various populations. Overall, these finding suggested that Thais population seemed be closely related to East Asian (Oriental).

Polymorphism of *NQO1* gene and cholangiocarcinoma risk

Cholangiocarcinoma (CCA) is a malignant tumor of the bile duct (Patel, 2006; Sirica, 2005) found with the highest incidence in the Northeastern region of Thailand (Vatanasapt et al., 1990). The reasons for the increasing occurrence of CCA are unclear. Several CCA risk factors, such as liver fluke, exposure to various chemicals or carcinogens, and individual genetic background, particularly polymorphisms found in phase I and II xenobiotic metabolizing enzymes (Prawan et al., 2005; Sripa et al., 2007), have been identified. However, the role of NQO1 genetic polymorphism in CCA has not been examined yet.

Up to date, there have been numerous studies examining the relationship between the *NQO1*2* polymorphism and cancer risk, but the results have been

contradictory for various cancers. Interestingly, in this current study, a higher *NQO1*1* allele frequency was detected in the CCA patients than that in the controls, on the other hand *NQO1*1/*2* (or heterozygote) genotype had a lower CCA risk than *NOQ1* wild type genotype. In addition, a greater increase of CCA risk was found in patients age ≥48 years with *NOQ1*1/*1* genotype, after adjusted for sex and smoking history. The age-related difference in CCA risk could be causally linked to the overwhelming accumulation of xenobiotic or carcinogens in elder. Surprisingly, data presented here did not show any enhanced effect of high cumulative dose of cigarette smoking in pack-years on CCA risk for the presence of the *NQO1*1/*1* genotype (p=0.06).

To our best knowledge, this is the first report of a negative association of the *NQO1*1/*2* genotype with CCA. Previously, several reports had been suggested that *NQO1*1/*1* was risk for cancers, such as lung cancer (Chen et al., 1999) and bladder cancer (Choi et al., 2003). Conflicting results, however, have also been reported. Lewis et al. (2001) found that UK patients carrying at least one *NQO1*2* allele had an almost four-fold increased risk of developing small cell lung cancer. Similarly, Park et al. (2003) observed that the *NQO1*2* carrying genotypes were associated with an increased risk of bladder cancer in Caucasians. These conflicting results, such as a positive association, weak-to-moderate association, no elevation of risk, and a negative association, may be several possibilities explained. 1) The fact that NQO1 may play either a protective role or a bioactivator enzyme depending on substrates/environmental exposures (Long et al., 2000; Ross et al., 2000). Possibly, the positive effect seen with the *NQO1*1/*1* genotype might well mean there were some potential bioactive and procarcinogenic compounds that hence predisposed individuals with higher NQO1 activity to have more CCA risk. 2) There may be other genetic mechanisms that compensate more effectively for the loss of the detoxifying activity of the *NQO1*2* allele genotype. 3) Furthermore, the *NQO1 C609T* polymorphism may be in linkage disequilibrium with other functional polymorphism. 4) In addition, the concept of genetic susceptibility is extremely complex and involves multiple cellular systems regulated by hundreds of genes, therefore, it is difficult to explain the association between genetic polymorphisms and risk of CCA by analyzing only one or two genes.

NQO1 genetic polymorphism in CCA patients and survival time

Etiologies of CCA have been suggested to be associated with various risk factors, including host (genetic) factor and environmental influence, as reported previously. Since there is a negative relationship between the *NQO1*2* allele and CCA risk, genetic polymorphism of this enzyme might be related to survival of CCA. Currently, there are few reports about NQO1 genetic polymorphism affecting survival time of cancer patient.

Our study is the first to investigate the role of the NQO1 polymorphism and CCA survival. However, we found no impact of the NOQ1 polymorphism on overall survival of CCA. Interestingly, Cox regression analysis showed that the papillary type was only a unique independent prognostic relevance in CCA in this study. In addition, we did not observed combination effect of *NQO1* genotype and papillary type on the survival time. Previously, there was a study reporting the ALL children having the *NQO1*2*, who were treated with a combination of chemotherapeutic agents, had shorter event-free survival compared to individuals with *NQO1*1* (p=0.003) (Krajinovic et al., 2002). Consistently other study (Fleming et al., 2002) showed that peritoneal cancer patients with *NQO1*2* have poorer prognosis than patients with *NQO1*1*. It should be noted that, gene-gene interaction in association with survival of CCA has not been clarified in this study. Moreover, other multifactors, such as the chemotherapy regimen of CCA patients, surgical procedure, other SNPs found in xenobiotic metabolizing enzymes, and also environmental exposures, need to be further clarified for their relations to CCA. Although, NQO1 polymorphism might not relate with survival time in CCA, the present study cannot exclude NQO1 as a possible candidate for CCA. Further study in a larger population and biological functional analysis of *NQO1* gene is required to verify the role of NQO1 in CCA.

In summary, the present study reported that polymorphism of NQO1 in Thais are of similar patterns as other Asian populations. Moreover, *NQO1*1/*1* genotype has been suggested to be associated with the increased risk of CCA in this Northeastern region of Thailand. However, we found no evidence that NQO1 genetic polymorphism is related to survival time. The knowledge from this work may be served as the preliminary data for more rigorous study determining the individual susceptibility to CCA development and the potential prognostic factors for CCA.