CHAPTER I
INTRODUCTION

1. Rationale and Background

NAD(P)H-quinone oxidoreductase-1 (NQO1), a flavoprotein mainly expressed in cytosol, catalyzes an obligate two-electron reduction of a broad range of substrates, particularly the most efficient substrate including quinines, quinone-imines, nitro and azo compounds. NQO1 enzyme requires a pyridine nucleotide cofactor, whereas the reduction reaction proceeds with equal facility with NADH or NADPH as a cofactor (Cullen et al., 2003; Jaiswal, 2000; Ross et al., 2000).

NQO1 plays an important role in the cellular detoxification by reducing quinones to more redox-stable hydroquinones in a single two-electron reduction. In contrast, metabolism by NQO1 may generate semiquinone radicals, which can undergo redox cycling in the presence of molecular oxygen leading to the formation of reactive oxygen species (Long et al., 2000; Ross et al., 2000). Interestingly, NQO1 plays a role in p53 stabilization by protection its from proteasomal degradation. Recently Anwar et al. (2003) proposed that NQO1 is able to physically associate with p53 suggesting that a protein-protein interaction may be responsible for the stabilization of p53 by NQO1. In addition, NQO1 is also capable of scavenging superoxide anions generated during oxidative stress and regenerating reduced forms of protective endogenous antioxidant compounds. A role for NQO1 as an antioxidant enzyme is further supported by recent immunohistochemical studies in humans that have shown expression of NQO1 protein in many tissues requiring a high level of antioxidant protection. These include the epithelial cells of lung, breast and colon, vascular endothelium, adipocytes, corneal and lens epithelium, retinal pigmented epithelium, optic nerve and nerve fibers (Siegel and Ross, 2000). The high levels of NQO1 suggest that NQO1 may function primarily in an antioxidant capacity of these cells. Surprisingly, NQO1 expression in human liver, a tissue with high degree of metabolic activity, is low where high level of NQO1 is found in biliary tissue. Interestingly, a dramatically up-regulation of NQO1 was found in many inflammatory conditions and tumorous tissues (Jaiswal, 2000; Strassburg et al., 2002).

Several studies have reported that the activity of the NQO1 enzyme strongly depends on polymorphisms at the *NQO1* locus. Although many single nucleotide polymorphisms have been characterized in NQO1 gene sequence, the major polymorphism associated with protein inactive is NQO1 P187S or *NQO1 * 2* allele. This mutation allele involves a single C to T substitution at nucleotide 609 of exon 6 that causes amino acid change from proline to serine at position 187. Therefore three NQO1 genotypes are classified, C/C (or *NQO1 *1/*1*), the homozygous wild-type phenotype with complete enzyme activity; C/T (or *NQO1*1/*2*), the heterozygous phenotype with ~3-fold decreased activity; and T/T (or *NQO1*2/*2*), the homozygous variant with a complete lack of enzyme activity. The other minor polymorphism is *NQO1 *3* allele which has a C to T substitution at nucleotide 465 that causes amino acid change from arginine to tryptophan at position 139. Comparing to the rare *NQO1*3* allele, the *NQO1*2* allele is much more frequent amongst different populations. Additionally, correlation of genotype and phenotype is well documented for *NQO1*2* allele. Available data indicate that the frequency of *NQO1*2/*2* genotype is predominant in Asians five-fold frequently than in Caucasians and four-fold than African-Americans (Zhang et al., 2003).

Interestingly, genetic polymorphisms of the NOQ1 enzyme that catalyze exogenous or endogenous carcinogens may determine individual susceptibility to the development of cancer. Up to date, many results concerning the association between NQO1 genetic polymorphism and risk of various cancers, such as lung cancer, bladder cancer, and colorectal cancer, have been reported. On the other hand, implications of NOQ1 polymorphism in neurodegenerative diseases are being investigated (Bian et al., 2008; Fong et al., 2007).

Induction of NQO1 activity has been well recognized as a consequence of various environmental exposures, including oxidative stress stimuli and antioxidant compounds, and can lead to increase defensive capacity against toxicity. Recently, the positive linkage between NQO1 induction and genoprotection has been suggested. However, *NQO1*2/*2* genotype with express only little NOQ1 protein resists to induction effect of the positive regulators, such as hydroquinone and benzoquinone. Therefore NQO1 may represent an interface between an external environmental

factors and genetic background. As a result, specific types of environmental exposure may lead individuals with genetic susceptibility to become prone to cancer.

Chronic infection and inflammation are important risk factors for several cancers, including cholangiocarcinoma (CCA), the primary cancer of the bile ducts (Lazaridis and Gores, 2005). The tumor arises from the ductular epithelium of the biliary tree within the liver (intrahepatic cholangiocarcinoma) or more common from the extrahepatic bile ducts (extrahepatic cholangiocarcinoma). The highest incidence of CCA worldwide has been reported in Northeast Thailand (Sriplung et al., 2006). The disease is notoriously difficult to diagnose and is usually fatal because of its late clinical presentation and the lack of effective non-surgical therapeutic modalities (Khan et al., 2005). *Opisthorchis viverrini* infection and primary sclerosing cholangitis (an inflammatory disease of the bile ducts) are important risk factors of CCA (de Groen et al., 1999). Inflammation of the biliary tract caused by mechanical injury and the release of metabolic products from the flukes, together with the damaging effects of reactive metabolites from endogenous and environmental chemicals have been proposed as the responsible factors, which induce alterations in gene expression resulting to cellular hyperproliferation and development of neoplasia. Thus, inflammatory processes which cause oxidative stress may affect NQO1 expression and inducibility. In addition, differences in NQO1 activity from genetic polymorphism may dispose an individual to cancer susceptibility.

In this study, we determined the polymorphism of *NQO1* gene in Thais and investigated the association between the NQO1 polymorphism and the risk of CCA development. We also evaluated the relationship between NQO1 polymorphism and survival times.

2. Hypothesis of the Study

2.1 There is an association between *NQO1*2* variant alleles and CCA risk. Since, the frequency of *NQO1*2* variant allele, which associated with reduced enzymatic activity, in CCA patients differs from that observed in control subjects, the *NQO1*2* variant allele can modify the risk of CCA.

2.2 The interindividual variation in the activity of NOQ1 could modify an individual's survival. As NQO1 functions to protect cells, the *NQO1*2* variant allele may alter the survival of CCA.

3. Aims of the Study

3.1 To determine the frequency of NQO1 polymorphism in Thai population and CCA patients.

3.2 To evaluate the association between NQO1 genotype and the risk of CCA and to assess the relationship between NQO1 genotype and survival times of the patients.

4. Specific Objectives

Blood specimens obtained from patients with CCA as a cancer groups, and healthy volunteers, as a control group were used.

4.1 To determine the frequencies of *NQO1* alleles in Thai population and compare its pattern of distribution with other populations.

4.2 To determine the association between the NQO1 genetic polymorphism and CCA risk.

4.3 To determine the relationship between the NQO1 genetic polymorphism and survival times in patients with CCA.

5. Scope of Thesis

The study investigated the relationship between the NQO1 genetic polymorphism and CCA. *NQO1* genotyping were performed to detect two *NQO1* alleles, including *NQO1*1* (wild-type) and *NQO1*2* (C609T). CCA patients recruited in this study were hospital based who had been undergone operation at Srinagarind Hospital, Khon Kaen University. Their diagnosis of CCA had been confirmed by tissue histopathology. Control subjects were healthy volunteers of Thai origin and lived in the Northeast Thailand. They did not have any previous diagnosis of any type of cancer.

6. Conceptual Framework

To elucidate the role of NQO1 in CCA, the genetic polymorphism of NQO1 in patients with CCA and control subjects were examined. The defective allele of NQO1 may associate with cholangiocarcinogenesis, in addition it may play role in poor prognosis or unresponsive response of chemotherapy.

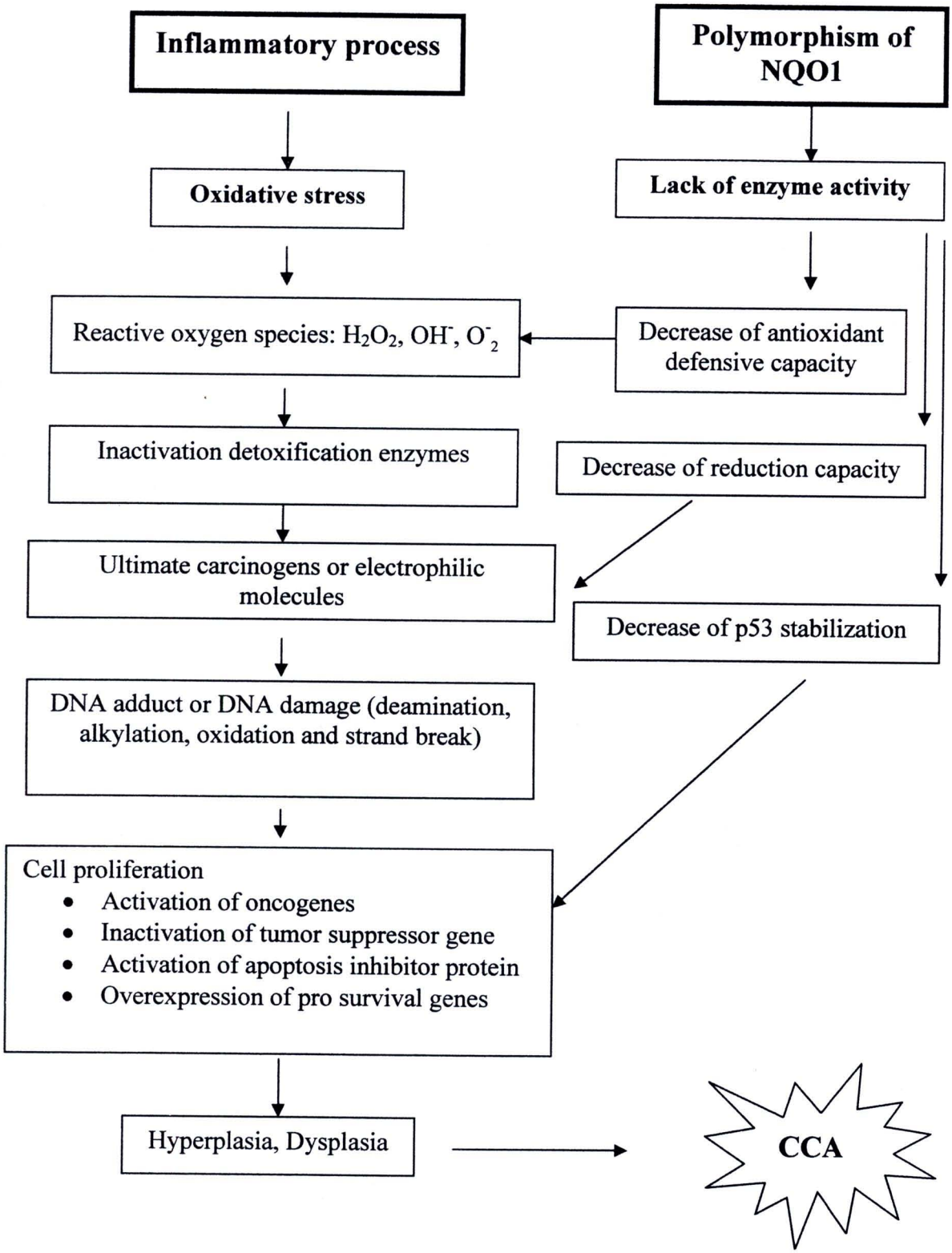

Figure 1 The conceptual framework of inflammatory on NQO1 activity, expression and impact of NQO1 polymorphism in CCA.