CHAPTER II
LITERATURE REVIEWS

1. Quinones, Redox cycling and Oxidative stress

Quinones are widely distributed in nature and constitute an important class of naturally occurring compounds. They are found in fungi, bacteria, plants, and vegetable (Jaiswal, 2000; Strassburg et al., 2002). Quinones of polycyclic aromatic hydrocarbons are abundant in all burnt organic materials, including automobile exhaust, cigarette smoke and urban air particulates. Compounds containing the quinoid nucleus are widely employed as antitumor agents (Workman, 1994). Overall, human exposure to quinones can occur via diet, airborne pollutants or clinically.

Quinones, including benzo(a)pyrene quinines and benzoquinones, are highly reactive molecules. They are also environmental carcinogens (Gelboin, 1980; Maltoni et al., 1983). Quinones undergo metabolism by either one- or two-electron reduction (Jaiswal, 2000; Strassburg et al., 2002). One-electron reduction of quinones and their derivatives, by enzymes such as cytochrome P450 reductase (P450 reductase), ubiquinone oxidoreductase, xanthine oxidoreductase, and cytochrome b5 reductase etc., generates unstable semiquinones which undergo redox cycling in the presence of molecular oxygen. This leads to the formation of highly reactive oxygen species (ROS) (e.g. superoxide anion, perhydroxyl radical, hydrogen peroxide, hydroxyl radical). The ROS and electrophiles (semiquinones) that are generated by one-electron reduction of quinones cause oxidative damage, such as binding to DNA, lipid peroxidation, and cytotoxicity (Jaiswal, 2000; Strassburg et al., 2002). The various toxic effects, caused by exposure to quinones, eventually lead to hepatic, cardiovascular, nervous and renal tissue degeneration, apoptotic cell death, premature aging, cellular transformation and neoplasia. Benzo(a)pyrene quinones are known to form DNA adducts and induce DNA strand breaks (Morrison et al., 1985). Therefore, quinones also contribute to benzene-induced toxicity and carcinogenicity.

In contrast, the two-electron reduction catalyzed by the NAD(P)H-quinone oxidoreductases-1 (NQO1) produces hydroquinones. These metabolites are more stable and can also be targeted for additional metabolism through conjugation with

glutathione or glucuronic acid. In promoting obligatory two-electron reduction, NOQ1 prevent the formation of reactive semiquinone intermediates. Thus, this enzyme plays an important role in cellular detoxification (Joseph et al., 2000; Lind et al., 1982; Thor et al., 1982) and, by preventing the generation of reactive oxygen species, has been considered as part of the human antioxidant defense system.

2. NAD(P)H-quinone oxidoreductase-1 (NQO1)

NAD(P)H-quinone oxidoreductase-1 (NQO1), a flavoprotein mainly expressed in cytosol, is an obligate two-electron reductase that is characterized by its capacity for utilizing either NADH or NADPH as a reducing cofactor and by its inhibition by dicoumarol. (Chao et al., 2006; Cullen et al., 2003; Jaiswal, 2000; Ross et al., 2000). NQO1 has attracted interest over the years as an enzyme involved in the detoxification of xenobiotics, such as quinones and quinoneimines, and an enzyme associated with protection against mutagenesis and carcinogenesis (Chesis et al., 1984; Lind et al., 1982; Thor et al., 1982). Recently, NQO1 has been characterized as being capable of generating antioxidant forms of ubiquinone and vitamin E after free radical attack (Li et al., 2006), providing conclusive evidence that this enzyme forms part of the body's antioxidant defense system. 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.

2.1 NQO1 substrate specificity

NQO1 is capable of reducing a very broad range of substrates, including quinones, quinone-imines, glutathionyl-substituted naphthoquinones, methylene blue, dichlorophenolindolphenol, azo, and nitro compounds. Both ortho and para quinones are substrates for NQO1 (Lind et al., 1990; Siegel et al., 1997). Metabolism is not limited to quinines and the enzyme functions efficiently as a nitro-reductase utilizing substrates, such as dinitropyrenes, nitrophenylaziridines, and nitrobenzamides.

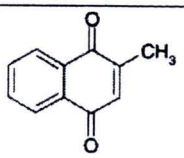
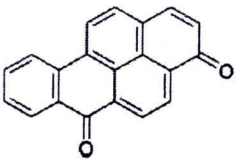
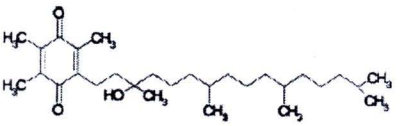
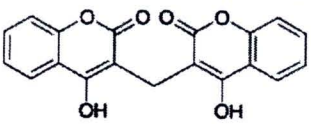
Compound	Comments	Structure
Menadione	Prototypical toxic redox-cycling agent and NQO1 substrate	
Benzo[*a*]pyrene-3,6-quinone	Toxic and mutagenic metabolite of benzo[*a*]pyrene. Generated by the cytochrome P450 system and detoxified by NQO1	
α-Tocopherol-quinone	Metabolite of α-tocopherol and potent lipid-soluble antioxidant. Maintained in reduced and active form by NQO1	
Dicoumarol	Inhibitor of NQO1 catalytic activity	

Figure 2 Structures of some substrates and inhibitors of NQO1 (Nioi and Hayes, 2004).

2.2 Function of NQO1 enzyme

2.2.1 Detoxification of substrates by two-electron reduction

The role of NQO1 as either a detoxification enzyme or an activation enzyme depending upon the stability of the hydroquinone generated following reduction. 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 (Figure 3) (Long et al., 2000; Ross et al., 2000). Another role for NQO1 in quinone detoxification is the removal of potentially arylating quinones. Quinones can readily undergo addition and substitution reactions and can react directly with protein thiols. Hydroquinone was selectively bioactivated in, and toxic to, marrow macrophages rather than fibroblasts in bone marrow stroma because of increased levels of peroxidases (leading to increased bioactivation) and lower levels of NQO1 (decreased deactivation) in macrophages relative to fibroblasts (Ganousis et al., 1992). Transfection of human promyeloblastic leukemia cells with NQO1 significantly decreased benzenetriol-DNA adduct formation (Wiemels et al., 1999).

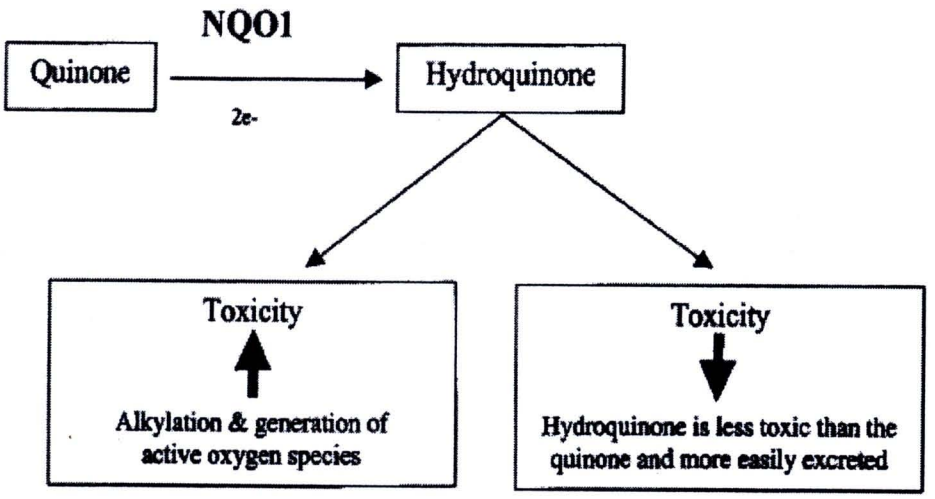


Figure 3 Activation and deactivation resulting from NQO1-mediated reduction of quinines (Ross et al., 2000).

2.2.2 NQO1 as an antioxidant defense enzyme

The NQO1 enzyme plays an antioxidant role via the reduction of endogenous quinones and these compounds, when reduced, help protect cellular membranes against oxidative damage (Figure 4) (Ross et al., 2000). Experiments have demonstrated that rat liver NQO1 can catalyze the reduction of ubiquinone analogs (coenzyme Q) to their ubiquinol forms in liposomes and rat hepatocytes (Landi et al., 1997). The rate of reduction of coenzyme Q derivatives was dependent upon the length of the carbon side-chain; short-chain homologs were reduced more efficiently than long-chains. In these studies it was shown that the ubiquinol formed following reduction by NQO1 was an effective antioxidant protecting membrane phospholipids from oxidative damage. α-Tocopherolquinone, a product of α-tocopherol (vitamin E) oxidation, has been shown to have antioxidant properties following reduction to α-tocopherolhydroquinone. An additional role for NQO1 in α-tocopherol metabolism has been postulated where NQO1 maintains physiological levels of α-tocopherol from the reduction of α-tocopherones by NQO1 (Cadenas et al., 1992).

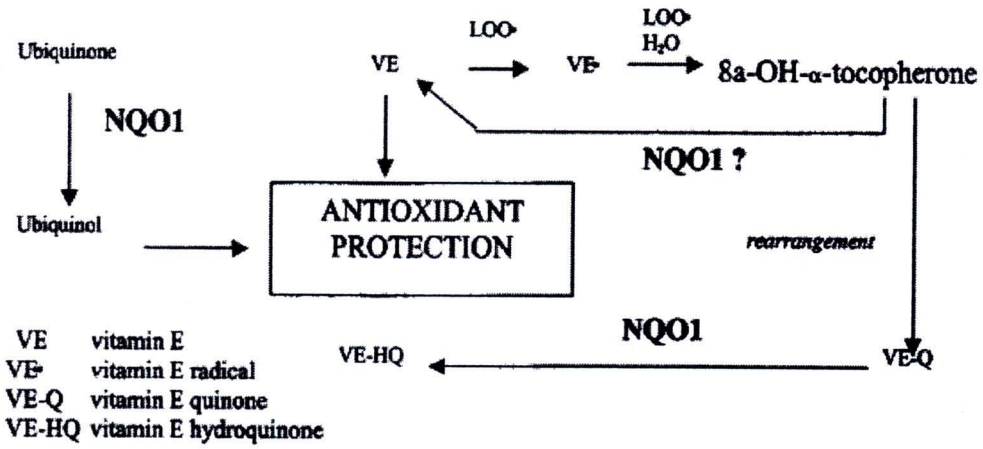


Figure 4 The role of NQO1 in regeneration of antioxidant forms of ubiquinone and vitamin E (Ross et al., 2000).

2.2.3 Stabilization of p53 tumor suppressor gene

Tumor suppressor p53 plays an important role in protection of genome against internal and external stresses. It prevents cell transformation and tumor formation through transcriptional dependent and independent mechanisms (Anwar et al., 2003; Sun, 2006). In response to a variety of stress signals, the p53 protein is stabilized and activated as a sequence specific transcription factor. This then leads to cell cycle arrest, senescence, or apoptosis (Asher et al., 2002).

NQO1 is physically associated with the 20S proteasomes and that NQO1 can also bind and protect a subset of short-lived proteins from 20S proteasomal degradation suggest that NQO1 may function as a gatekeeper of the 20S proteasomes in the cells (Gong et al., 2007). Asher and Shaul, (2006) propose a model whereby some short-lived proteins (such as p53, p73 and ODC) are inherently unstable and degraded "by default" by the 20S proteasomes in cells unless stabilized by a stabilizer, such as NQO1. This degradation by default mechanism is distinct from the current "modification to destabilization" mechanism that is mediated by poly-ubiquitination. The nascent protein that manages to escape 20S proteasomal breakdown matures and is engaged in larger functional protein complexes. At this stage, the degradation of the protein can occur mostly via ubiquitin-dependent 26S proteasomal degradation.

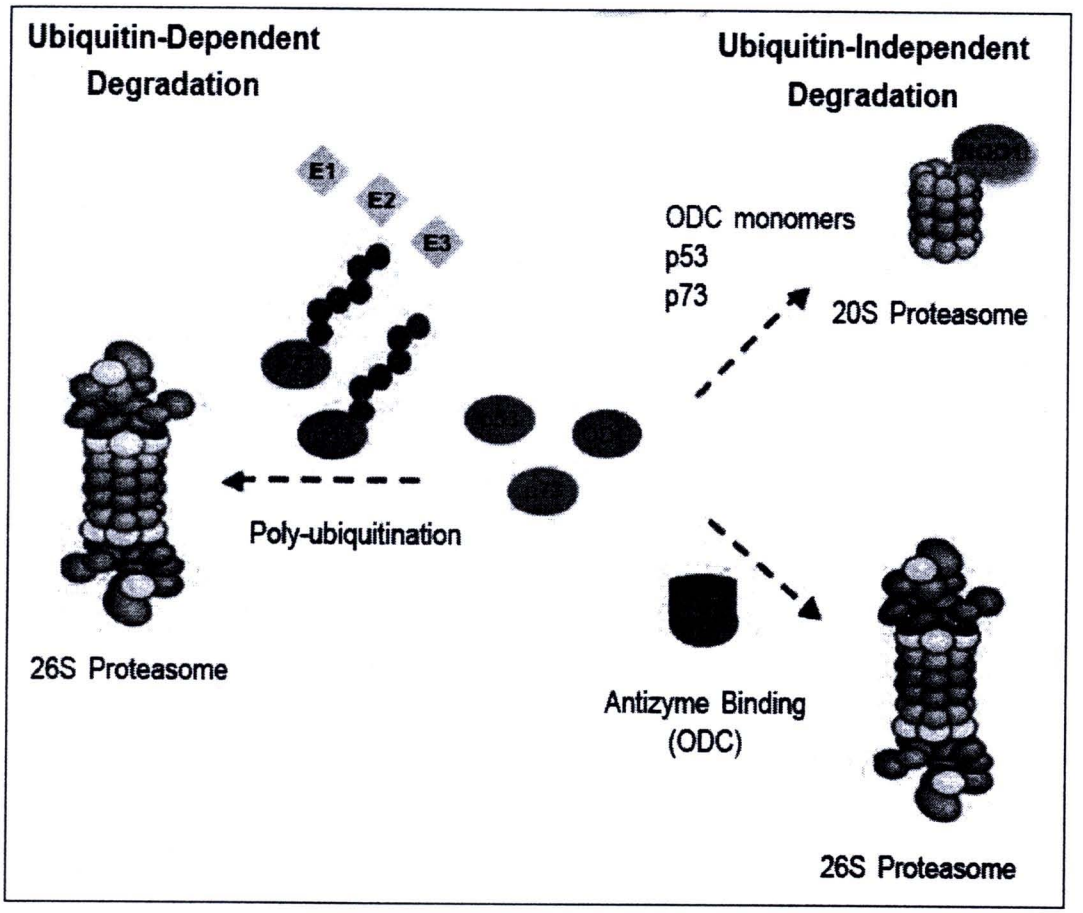


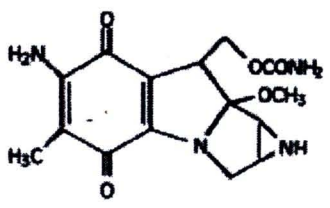
Figure 5 Schematic representation of ubiquitin-dependent and independent mechanisms for proteasomal protein degradation (Asher and Shaul, 2006).

2.3 Bioactivation by NQO1 in chemotherapy: Enzyme-directed antitumor agents

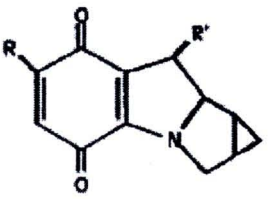
Drugs that can produce alkylating metabolites after reduction have been termed 'bioreactive alkylating agents'. The result of bioreduction is either the production of alkylating species or active oxygen species depending on the chemical properties of the compounds undergoing enzymatic reduction (Ross et al., 2000).

Enzyme-directed antitumor drug development, however, exploits bioactivating enzymes that are expressed at high levels in tumors relative to uninvolved tissue. NQO1 is expressed at high levels throughout many human tumors, such as bladder cancer (Moore et al., 2004; Park et al., 2003), lung cancer (Alexandrie et al., 2004), esophageal squamous cell carcinoma (Zhang et al., 2003), prostatic

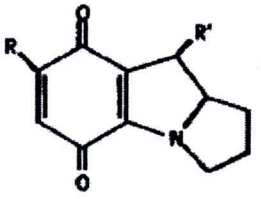
adenocarcinama (Steiner et al., 1999), and is one possible candidate for the enzyme-directed approach. Since NQO1 is present in uninvolved tissues as well as human tumor tissue (Jaiswal, 2000), it is possible that toxicity to normal tissue may be an issue in therapy with NQO1-directed antitumor quinones.



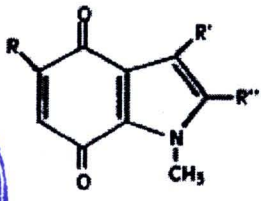
Mitomycin C



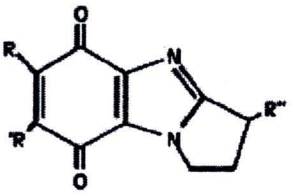
Cyclopropamitosene



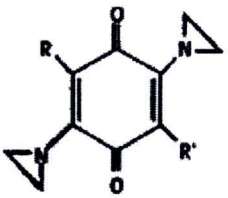
Mitosene



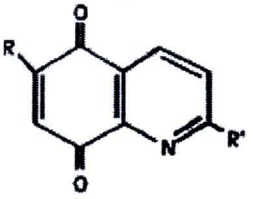
Indolequinone



Pyrrolobenzimidazolequinone



Aziridinylbenzoquinone



Quinolinequinone

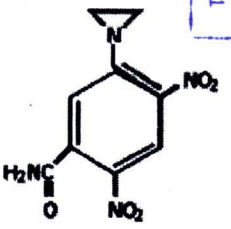


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Figure 6 Quinones and other compounds considered as NQO1-directed antitumor agents (Ross et al., 2000).

Mechanistically, DNA is thought to be the target of such enzyme-directed alkylating agents, so the higher growth fraction of tumors may still offer opportunity for selective toxicity. The development of quinones which can be efficiently bioactivated by NQO1 as potential antitumor agents has focused on

aziridinylbenzoquinones, indolequinones, motosenses, pyrrolobenzimidazolequinones, and cyclopropamitosenses (Figure 6).

Mitomycin C (MMC) is currently used in combination cancer therapies. The bioreductive activation of the antitumor quinone MMC by NQO1 is complicated by the ability of MMC to also act as a mechanism based inhibitor of NQO1 in a pH dependent manner, and this pH dependence seems to be due to inactivation of NQO1 by MMC at more neutral pH (Gustafson et al., 2003). MMC has been designed in the hopes of generating more efficient substrates and therefore better chemotherapeutic agents. In structure–activity relationship studies using indolequinones and mitosenes, a number of features have been identified which impact metabolism and cytotoxicity, and these have been recently summarized (Gustafson et al., 2003). Depending on the substitution patterns in the indole or mitosene structure, compound design within the structural series can be optimized for potency and selective toxicity to cells containing high NQO1 levels.

MeDZQ (2,5-diaziridinyl- 3,6-methyl-1,4-benzoquinone) metabolism by NQO1, and cytotoxicity and selectivity to NQO1-containing cells was also increased. Pyrrolobenzimidazolequinones (PBIs) were designed to alkylate the phosphate backbone of DNA upon reduction which results in its cleavage. PBI substrates for NQO1 can be either activated or deactivated by the enzyme depending on their structure (Skibo et al., 1997). Quinolinequinones and benzoquinone mustards have also been considered as potential agents for NQO1-directed approaches to chemotherapy (Ross et al., 2000).

2.4 NQO1 regulation

NQO1 is a single copy gene that located on human chromosome 16q22.1 (Jaiswal, 2000; Ross et al., 2000). Restriction mapping and sequencing have revealed that the NQO1 gene consists of six exons and five introns for an approximate length of 20 kb (Chen et al., 1991; Jaiswal, 1991; Shaw et al., 1991). Exon 1 encodes the first two amino acids and the first nucleotide of the third amino acid, while exons 2–6 encode the remaining 272 amino acids.

Among several regulatory elements found in the NQO1 promotor region, two distinct regulatory elements that have been studied extensively are the antioxidant response element (ARE), also called the EpRE (electrophile response

element), and the xenobiotic response element (XRE), also called the AhRE. The ARE and the XRE have been shown to mediate NQO1 induction as well as repression, in many cellular systems. The structure–function relationships within the NQO1 promoter are now being addressed using functional assays, mutational analysis, and transgenic models (Chen et al., 1991; Jaiswal, 1991; Ross et al., 2000).

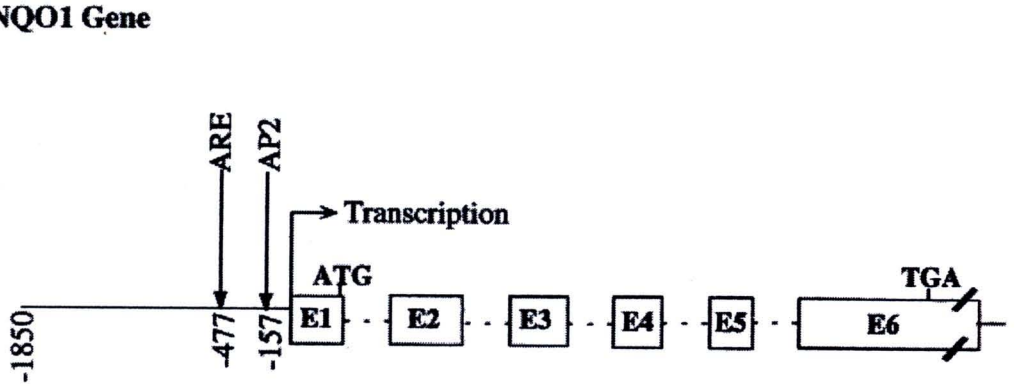

Figure 7 *NQO1* gene structure and promoter elements. Structure of human *NQO1* gene; E1-E6 Exons; ARE, antioxidant response element, AP-2, AP-2 binding site (Jaiswal, 2000).

2.4.1 Induction of NQO1 by antioxidants

A hypothetical model shows ARE mediated induction of NQO1 and other detoxifying enzyme genes expression in response to xenobiotics and antioxidants (Jaiswal, 2000; Li and Jaiswal, 1994; Long et al., 2000; Prestera et al., 1993). The other genes that are coordinately induced with the NQO1 gene include glutathione *S*-transferases (GSTs), which conjugate hydrophobic electrophiles and reactive oxygen species with glutathione (GSH); UDP-glucuronosyl transferases (UDP-GT), which catalyze the conjugation of glucuronic acid with xenobiotics and drugs for their excretion; epoxide hydrolase (EH), which inactivates epoxides; γ-glutamylcysteine synthetase (γ-GCS), which plays a key role in the regulation of glutathione metabolism, and so on. The coordinated induction of these genes,

including NQO1, presumably provides necessary protection for cells against free radical damage, oxidative stress, and neoplasia (Long et al., 2000).

2.4.2 Up-regulation of NQO1 by inflammation and oxidative stress

NQO1 gene expression in response to cytokine, tumor promoters, H_2O_2 , $O_2°$, heavy metals, UV light, and ionizing radiation, has been reported (Jaiswal, 2000; Li and Jaiswal, 1994; Long et al., 2000; Prestera et al., 1993). Xenobiotics and antioxidants undergo metabolism to generate superoxide and electrophiles (Pinkus et al., 1996). The generation of superoxide and related species is a common phenomenon between antioxidants and xenobiotics. For this reason, superoxide is believed to serve as signal that activates a battery of defensive genes, including NQO1, that protect cells against the adverse effects of oxidative stress. Hydrogen peroxide, however, has been demonstrated to induce the ARE mediated expression of rat GST Ya, rat NQO1, and human NQO1 genes (Favreau and Pickett, 1991).

The superoxide signal presumably passes either directly or through unknown intermediary proteins to the cytosolic factor(s) (Figure 8). The cytosolic factor(s) catalyze modification of Nrf2 leading to the dissociation of Nrf2 from Keap1. Nrf2 translocates into the nucleus. Alternatively, cytosolic factor(s) catalyze modification of Keap1 resulting in dissociation of Nrf2 from Keap1. This follows the translocation of Nrf2 in the nucleus. Nrf2 after translocation in the nucleus forms heterodimer with c-Jun, which binds to the ARE resulting in the induction of NQO1 and other ARE-regulated genes expression. C-Jun may also undergo some modifications by cytosolic factor(s) before moving to the nucleus for heterodimerization with Nrf2. However, this is less likely because c-Jun expression is significantly increased in response to xenobiotics and antioxidants (Jaiswal, 2000). Nrf1 is expected to function in a similar manner as Nrf2. The Jun-B and Jun-D may function similar as c-Jun. This is because of their role in ARE mediated expression and induction of NQO1 gene expression (Venugopal and Jaiswal, 1998).

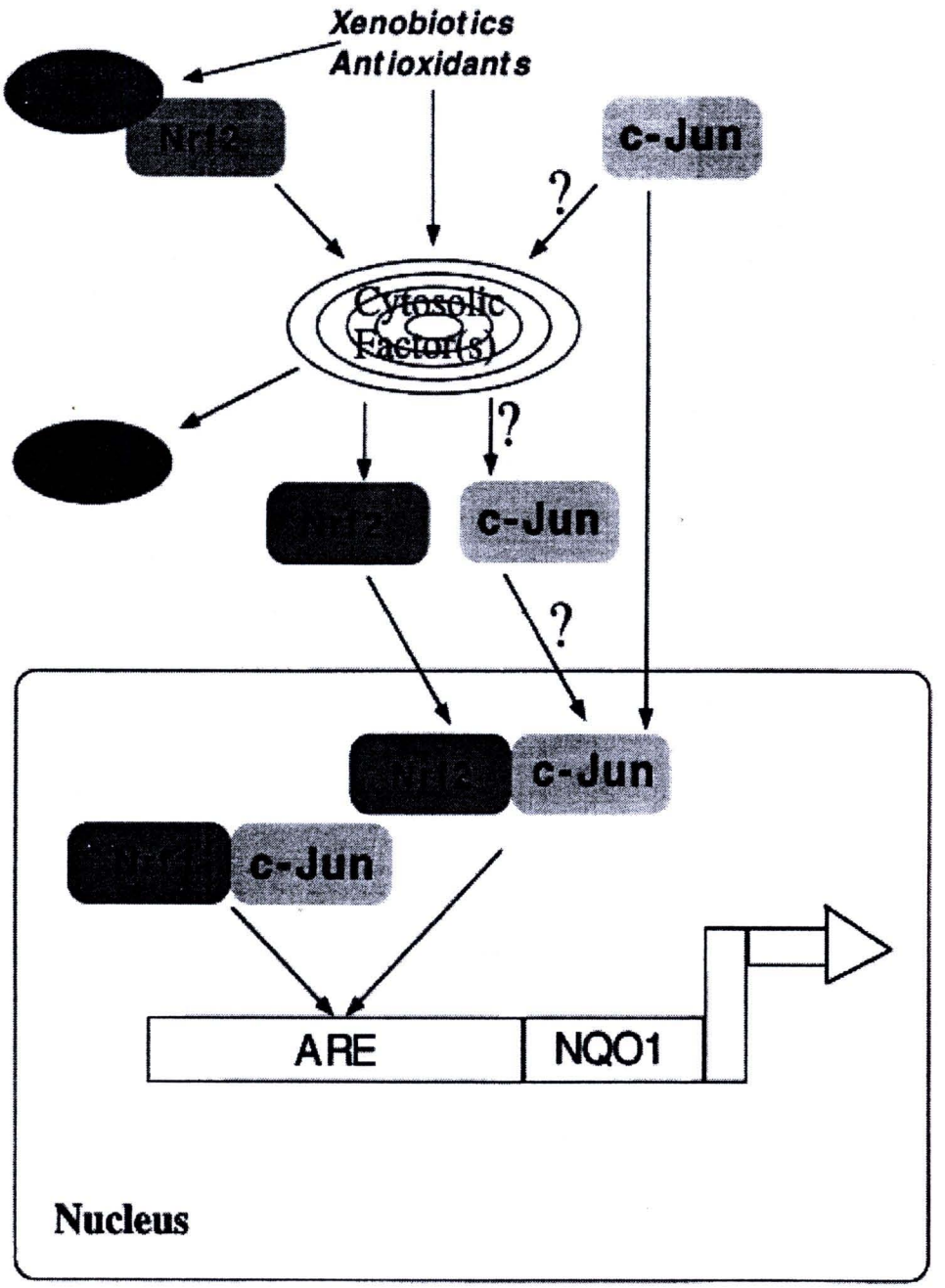

Figure 8 Transcription factors recruited to the NQO1-ARE under homeostatic conditions or during oxidative stress (Jaiswal, 2000).

2.5 NQO1 expression

Human Northern blot analysis using *NQO1* cDNA probe indicated differential expression of this gene in the heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. The *NQO1* gene expression was highest in kidney followed by skeletal muscle and lung. The pancreas and brain showed minimal expression of the *NQO1* gene (Jaiswal, 1991). Moreover, a dramatically up-regulation of NQO1 was found in a variety of solid tumors. Marked NQO1 staining was detected in solid tumors from thyroid (Siegel and Ross, 2000), bladder (Moore et al., 2004; Park et al., 2003), breast, ovarian (Siegel and Ross, 2000), colon (Chao et al., 2006; Mitrou et al., 2007), non-small cell lung cancer (Alexandrie et al., 2004; Chao et al., 2006), esophageal squamous cell (Zhang et al., 2003), prostate (Steiner et al., 1999), and in both malignant hepatocellular and biliary tissues (Strassburg et al., 2002). These finding are consistent with Jaiswal et al. (2000) proposed that NQO1 is up-regulated in many inflammatory conditions and solid tumors.

2.6 NQO1 polymorphism

2.6.1 Genotype-phenotype relationships

Several studies have reported that the activity of the NQO1 enzyme strongly depends on polymorphisms at the *NQO1* locus (Gaedigk et al., 1998; Kelsey et al., 1997; Misra et al., 1998). Although there are many single nucleotide polymorphisms (SNPs) have been characterized, only two SNPs in NQO1, the *NQO1*2* polymorphism and the *NQO1*3* polymorphism, affect the encoding of amino acid sequence. However, the allele frequency of the *NQO1*3* polymorphism is low, and the phenotypic consequences are variable according to substrate (Hu et al., 1996; Pan et al., 1995).

The *NQO1*2* polymorphism is a single nucleotide change at position 609 of the *NQO1* cDNA coding for a proline to serine change at position 187 in the amino acid structure of the protein. This polymorphism has significant phenotype consequences and results in a lack of NQO1*2 protein in tissue samples from individuals carrying the homozygous *NQO1*2* polymorphism. Comparisons between the *NQO1*1/*1* genotype and the *NQO1*2/*2* genotype demonstrated similar levels of mRNA, similar mRNA half-lives in cells, and similar rates of transcription and translation in a coupled cell-free system. These data suggested that the defect with

respect to NQO1 expression was at the level of the protein. Indeed, the mutant NQO1*2 protein was found to be degraded rapidly by the ubiquitin proteasomal system with a half-life of approximately 1.5 h. The wild type protein is stable in cells for periods longer than 18 h so the homozygous *NQO1*2* polymorphism confers a major phenotypic change. Individuals heterozygous for the *NQO1*2* polymorphism (i.e. *NQO1*1/*2* genotype) have amounts of protein and activity levels intermediate between *NQO1*1/*1* (large amounts of stable protein) and *NQO1*2/*2* (no protein detected in tissues, and trace levels of protein detected in cultured cell systems). Since the NQO1 gene is highly inducible, however, there can be a considerable range of NQO1 levels in both wild-type and heterozygous individuals (Ross, 2005).

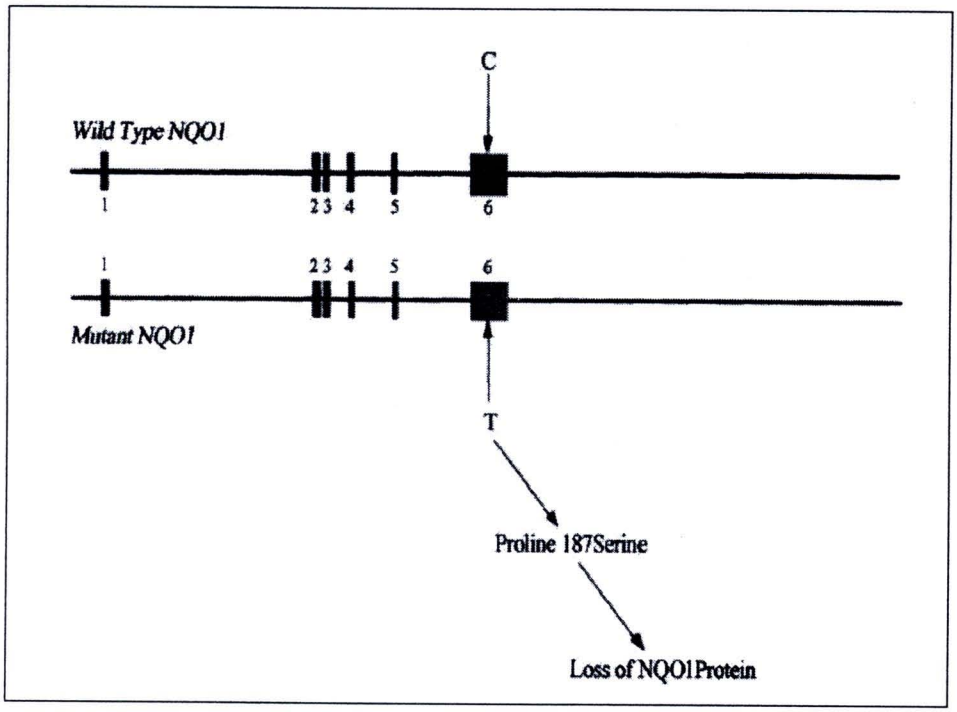

Figure 9 Human *NQO1* gene polymorphism (Ross et al., 2000).

Table 1 Percentage of individuals in different populations with the *NQO1*2/*2* genotype.

Population	n	*NQO1*1* allele (%)	*NQO1*2* allele (%)	*NQO1*2/*2* (%)
Caucasian (Saldivar et al., 2005)	683	84	16	2.49
African-American (Saldivar et al., 2005)	107	81	19	2.80
Hispanics (Moore et al., 2004)	108	53	47	6.5
Korean (Choi et al., 2003)	170	46	54	9.4
Chinese (Zhang et al., 2003)	165	57.6	42.4	16.35
Japanese (Hori et al., 2003)	204	60	40	17.2
Hmong (Kiffmeyer et al., 2004)	198	39	61	34

The prevalence of the *NQO1*2/*2* genotype (Table 1) varies in different ethnic groups, but is as high as 17.2 % in Japanese populations (Hori et al., 2003). Recent work has reported an even higher prevalence of the homozygous *NQO1*2/*2* genotype in ethnic Hmong populations living in the US of 34% (Kiffmeyer et al., 2004). Interestingly, the prevalence of the *NQO1*2/*2* genotype in Caucasians is in agreement with an early phenotype study published many years prior to characterization of the *NQO1*2* polymorphism.

2.6.2 NQO1 polymorphism and Cancer susceptibility

2.6.2.1 Solid tumors: Pancreatic cancer, lung cancer, bladder cancer, colorectal cancer

Previous work on the implications of the *NQO1*2* or null polymorphism in NQO1 have almost exclusively been examined from the perspective of the susceptibility to cancer of individuals carrying the *NQO1*2* allele. The *NQO1*2/*2* genotype may affect individual susceptibility to many solid tumors.

Pancreatic cancer, (Lyn-Cook et al., 2006), reported the increased NQO1 expression in non cancer pancreatic tissue from smokers, and the fact that smoking is a moderate risk factor for pancreatic cancer suggest that NQO1 expression may be a good candidate as a biomarker for pancreatic cancer, especially in risk groups, such as smokers. The effect of the genetic polymorphism of NQO1 on the risk of pancreatic diseases (cancer, pancreatitis). (Bartsch et al., 1998), the distribution of frequencies for NQO1 genotypes did not differ in subjects with pancreatic diseases compared with controls. The results of this study, requiring confirmation, suggested that the polymorphism of NQO1 may be associated with a modest increase in susceptibility to pancreatic diseases.

Lung cancer, Sardivar et al. (2005) reported the *NQO1*2* allele was associated with a marginally increased lung cancer risk (OR = 1.19; 95% CI: 0.95–1.50). This result is also consistent with Alexandrie et al. (2004), the *NQO1*2* allele was significantly associated with increased risk of squamous cell carcinoma (OR = 1.16; 95% CI: 0.72–1.88).

Bladder cancer, Moore et al. (2004) reported the *NQO1*2/*2* allele were associated with increased bladder cancer risk (OR = 1.26; 95% CI: 0.44–3.61). This is similar to Park et al. (2003) reported, the variant allele

genotypes of NQO1 were associated with higher bladder cancer risk (OR = 1.51; 95% CI: 1.01–2.85).

Colorectal cancer, Mitrou et al. (2007) reported the *NQO1*2* allele was positively associated with high risk sporadic distal colorectal adenomas (OR = 1.36; 95% CI: 1.02–1.83).

Interestingly, there are many data up to date showed different of individuals frequency in populations with the *NQO1*2/*2* genotype. In addition, many studies also demonstrates the effects of the NQO1 polymorphism seem to be modified by ethnicity and environment exposure, such as smoking status, age or sex.

2.6.2.2 Hematologic malignancies: Leukemias

There are epidemiological studies associating the *NQO1*2* polymorphism with an increased risk of leukemia. (Smith et al., 2002) reported patients with de novo leukemias with MLL translocations were significantly more likely to be *NQO1 *1/*2* genotype (OR = 2.77, 95%CI: 1.17-6.57). In secondary malignant neoplasm (SMN) therapy, (Stanulla et al., 2007) reported the *NQO1*2* allele was a significantly increased risk of developing a SMN (OR = 2.43, 95%CI: 1.04-5.70). Chronic lymphocytic leukemia (CLL), (Begleiter et al., 2008) reported patients with CLL were significantly more likely to be *NQO1 *2/*2* genotype (OR = 1.41, 95%CI: 0.54-3.70).

2.6.3 NQO1 polymorphism and Neurodegenerative diseases

Large amounts of neurotoxic quinone intermediates derived from dopamine metabolism are key to generation of oxidative stress, leading to the development of neurodegenerative disorders, such as Alzheimer's disease (Bian et al., 2008), Parkinson's disease (Fong et al., 2007), and schizophrenia (Hori et al., 2003). NQO1 protect cell from ROS damage during redox cyclic procedure. Therefore, previous work indicated the *NQO1*1* allele is a possible protective effect against neurodegenerative development, and the *NQO1*2* allele might be a weak risk factor for the development of neurodegenerative disorders. However, the association of this gene polymorphism and neurodegenerative disorder may be cofactor with other genes (Bian et al., 2008).

Table 2 NQO1 polymorphism and cancer susceptibility

Cancer	Population	N.	NQO1 genotype	OR (95% CI)	Risk factor
Lung cancer					
(Saldivar et al., 2005)	Caucasian	826	*1/*2, *2/*2	1.19 (0.95-1.50)	Sex, age, smoking status
(Alexandrie et al., 2004)	Sweden	312	*1/*2, *2/*2	1.16 (0.72-1.88)	Age, smoking status
Bladder cancer					
(Moore et al., 2004)	Argentina	106	*2/*2	1.26 (0.44-3.61)	Age, smoking status
(Park et al., 2003)	Caucasian	265	*1/*2, *2/*2	1.51 (1.01-2.25)	Sex, age, smoking status
Cololectal adenomas					
(Mitrou et al., 2007)	UK	894	*1/*2, *2/*2	1.36 (1.02-1.83)	Age, smoking status, alcohol
Secondary malignant neoplasm (SMN)					
(Stanulla et al., 2007)	Germany	78	*1/*2, *2/*2	2.43 (1.04-5.70)	Age, sex
Chronic lymphocytic leukemia (CLL)					
(Begleiter et al., 2008)	Canada	323	*2/*2	1.41 (0.54-3.70)	Age, sex
MLL					
(Smith et al., 2002)	UK	39	*1/*2	2.77 (1.17-6.57)	Age, sex

Table 3 NQO1 polymorphism and low risk of cancer

Cancer	Population	N.	NQO1 genotype	OR (95% CI)	Risk factor
Lung cancer					
(Skuladottir et al., 2005)	Denmark & Norway	320	**1/*2, *2/*2*	0.74 (0.47-1.14)	Sex, age
(Lan et al., 2004)	Taiwan	198	**1/*2, *2/*2*	0.91 (0.51-1.62)	Sex, pack-year smoked
(Hamajima et al., 2002)	Japanese	192	**1/*2*	0.71 (0.50-1.00)	Sex, age
(Chen et al., 1999)	Japanese	327	**2/*2*	0.80(0.40-1.50)	Sex, age, smoking status, saturated fat, vegetable intake
Bladder cancer					
(Broberg et al., 2005)	Sweden	61	**1/*2*	0.72 (0.34-1.50)	Sex, age, smoking status
(Choi et al., 2003)	Korean	218	**1/*2, *2/*2*	0.63 (0.37-1.00)	Age, urinary track stones, smoking status
Esophageal adenocarcinoma					
(di Martino et al., 2007)	UK	327	**2/*2*	0.22 (0.07-0.76)	Sex, age, smoking status, body mass index, reflux system

2.6.4 The protective effect of the NQO1 variant genotype

Several studies found that the NQO1 C609T polymorphism seems to modify the risk of many cancers, such as lung cancer, bladder cancer, and Barrett esophagus adenocarcioma.

A clear association between the polymorphism and lung cancer risk was not indicated in whites. It seems that in Asian populations, the polymorphism may possibly decrease the risk of lung cancer. An inverse dose-response was observed with increasing numbers of the T allele, providing further support of the causal association between the NQO1 C609T polymorphism and lung cancer in Asians. Hamajima et al. (2002) reported the *NQO1*2* allele was associated with decreased lung cancer risk (OR = 0.71; 95% CI: 0.56–1.00). These results were also consistent with Lan et al. (2004) the *NQO1*2* allele was significantly associated with low risk of lung cancer (OR = 0.91; 95% CI: 0.51–1.62).

For bladder cancer, the presence of the variant genotype seems to increase risk, and indeed, the relative risk is greater among those who are homozygous than those who are heterozygous. Broberg et al. (2005) reported the *NQO1*1/*2* allele was associated with decreased bladder cancer risk (OR = 0.72; 95% CI: 0.34-1.50). These are similar to Choi et al. (2003) reported, the variant allele genotypes of *NQO1* were associated with lower bladder cancer risk (OR = 0.63; 95% CI: 0.37–1.00).

In Barrett esophagus adenocarcioma, di Martino et al. (2007) reported the *NQO1*2* allele were associated with low risk (OR = 0.22; 95% CI: 0.07–0.76). Interestingly, there are many data up to date showed the potential protective effect of the variant genotype in Asians which may be explained by the failure of the variant enzyme to bioactivate procarcinogens, such as nitrosamine in tobacco. Such reduced activity in activating environmental procarcinogens seems to be more important than the reduced detoxifying activity in Asian populations in modifying cancer risk

3. Cholangiocarcinoma (CCA)

3.1 Definition and Classification

Cholangiocarcinoma (CCA) is a malignant tumor of the bile duct, that originates from the bile duct epithelial cell at any portion of the biliary tree from the level of bile duct at the ampulla of Vater (Lazaridis and Gores, 2005; Patel, 2006; Sirica, 2005). More than 90% of CCA are adenocarcinomas (Wu, 2005). 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 (Lim, 2003).

According to its location in the biliary tree, CCA is classified into extrahepatic and intrahepatic types (Figure 10) (Lazaridis and Gores, 2005). The extrahepatic type accounts for approximately two thirds of all CCA and is further divided into: (1) hilar or Klatskin, (2) middle, and (3) distal tumors. Klatskin tumors represent approximately 60% of all extrahepatic CCA. Macroscopically, extrahepatic CCA presents as sclerosing, nodular, or papillary phenotypes. The sclerosing type is the most frequent and results in annular thickening of the bile ducts because of infiltration and fibrosis of the periductal tissues. The intrahepatic CCA are classified into the following 4 growth types: (1) mass forming, (2) periductal infiltrating, (3) mass forming plus periductal infiltrating, and (4) intraductal (Lazaridis and Gores, 2005).

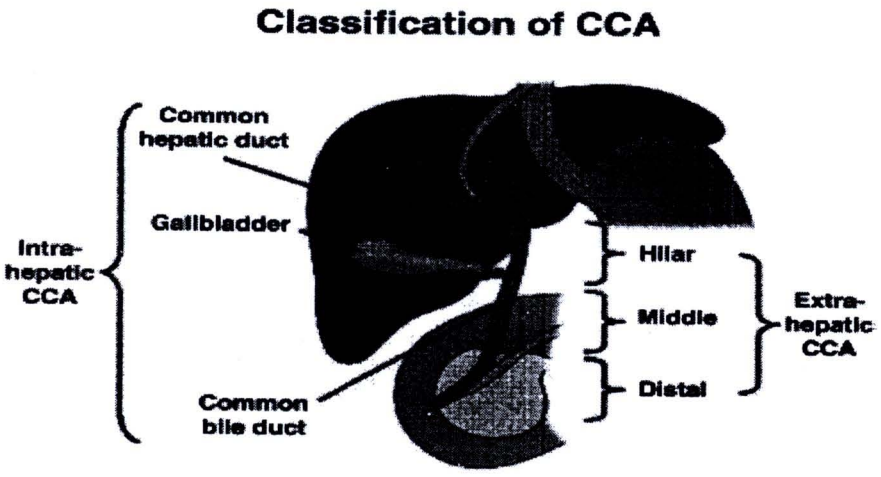

Figure 10 The term CCA refers to tumors involving the entire (ie, intrahepatic and extrahepatic) biliary tree (Lazaridis and Gores, 2005).

Incidence rates for intrahepatic CCA vary substantially worldwide, reflecting the distribution of local geographic risk factors (Nakanuma et al., 1997; Tullo et al., 2000). The incidence of extrahepatic CCA has been found less than intrahepatic CCA, the most common cases in Northeast Thailand, Laos, and China (Nakanuma et al., 1997; Wu, 2005).

3.2 Epidemiology

The incidence rate of CCA in Western countries is very low with appoximately 2 case per 100,000 people. On the other hand, the incidence rate of CCA in Asia countries (e.g. Thailand, Southern China, Korea, and Hong Kong) is estimated to be 50 times higher than that in Western countries (Sripa et al., 2007; Sriplung et al., 2006; Upatham and Viyanant, 2003; Uttaravichien et al., 1999). CCA are uncommon cancers in the United States (Okuda et al., 2002). There are estimated 7,000 new case of gallbladder cancer and CCA diagnosed annually in the United States, with approximately 3,500 deaths in the same period (Jemal et al., 2003). The mortality rate from CCA has been on the rise in the United States and other countries. In the Northeast Thailand, the incidence rate of CCA is 89.5 cases per 100,000 males and 35.5 cases per 100,000 females with age standardized annually (Vatanasapt et al., 2002).

Most of CCA in Western countries associated with the pathology of the bilialy system or related diseases, such as primary sclerosing cholangitis or chronic ulcerative colitis. In contrast, CCA in Thailand is mostly associated with the liver fluke, *Opisthorchis viverrini*, infection (de Groen et al., 1999). This indicates that the etiologies of CCA in two regions are different. The reasons for the increasing occurrence of CCA are unclear. The improvement of diagnostic methods may be partially responsible, but the prevalence of potential risk factors for CCA, such as HIV infection, has also been increasing during this time frame (Shaib et al., 2005).

3.3 Etiology

CCA are sporadic, and no precipitating factor can be identified (Gores, 2003). CCA occurs at a younger age, and approximately one-third of cancers are diagnosed within 1 year of diagnoses of primary sclerosing cholangitis. Primary sclerosing cholangitis (PSC), is an autoimmune condition strongly associated with ulcerative colitis. PSC result in inflammation of the bile duct. Subsequence chronic

disease can lead to multifocal strictures of the biliary system (Angulo and Lindor, 1999). In addition choledochal cysts induced bile stasis in the cysts also leads to chronic inflammation of the duct and great increased risk of CCA.

Liver fluke infestation is estimated to afflict 17 million people worldwide, mostly in Southeast Asia and China. These parasites lead to chronic inflammation of bile ducts. The incidence of CCA in these countries ranks among the highest in the world (87 per 100,000). The liver flukes response for CCA are *Opisthorchis viverrini* and *Clonorchis sinensis*. Liver fluke, *Opisthorchis viverrini*, infection has been classified to be human carcinogenic agent according to International Agency for Research in Cancer (IARC).

3.4 Pathology

CCA can affect any area of the bile ducts, either within or outside the liver. Tumors occurring in the bile ducts within the liver are referred to as intrahepatic; those occurring in the ducts outside the liver are extrahepatic, and tumors occurring at the site where the bile ducts exit the liver may be referred to as perihilar. A CCA occurring at the junction where the left and right hepatic ducts meet to form the common bile duct may be referred to eponymous as a Klatskin tumor. The cell of origin of CCA is unknown, although recent evidence has suggested that it may arise from a pluripotent hepatic stem cell (Liu et al., 2004; Roskams, 2006). CCA is thought to develop through a series of stages from early hyperplasia and metaplasia, though dysplasia, to the development of frank carcinoma in a process similar to that seen in the development of colon cancer (Sirica, 2005). Chronic inflammation and obstruction of the bile ducts, and the resulting impaired bile flow, are thought to play a role in this progression (de Groen et al., 1999; Holzinger et al., 1999; Sirica, 2005).

3.5 Mechanism of Cholangiocarcinogenesis

Carcinomas of the biliary tract are rare cancers developing from the epithelial or blast-like cells lining the bile ducts. A variety of known predisposing factors and recent experimental models of biliary carcinogenesis (e.g. infection with the liver fluke *Opisthorchis viverrini*, models of chemically induced carcinogenesis, and experimental models of pancreaticobiliary maljunction) have been elucidated at different stages of this complex system of biliary tumorigenesis.

Chronic inflammation induced by *Opisthorchis viverrini* infection associated with cytokines, namely TNF-α and interleukin-γ (IL-γ), is produced by activated macrophage, kupffer cells, and hepatocytes during inflammation. Macrophages activated by such cytokines produce nitric oxide (NO) and its metabolites, which have been shown to induce cytotoxic and mutagenic effects in target cells when present in excess (Ohshima et al., 1994). NO and related oxygen radicals can activate and injure the biomolecules within the cells, especially DNA. The activation of inducible NOS (iNOS) and excessive NO production in response to inflammatory cytokines causes oxidative DNA damage and inactivation of DNA repairing proteins (Jaiswal, 2000).

Recently, It has been demonstrated that 8-oxo-7, 8-dihydro-2'-deoxyguanosine and 8-nitroguanine are biomarkers for DNA damage in the liver of *Opisthorchis viverrini* infected hamsters and suggested that these oxidative and nitrosative DNA damage and iNOS expression induced by *Opisthorchis viverrini* infection via inflammation might play a key role in the initiation and promotion step of CCA development (Pinlaor et al., 2004). Moreover, inflammatory cytokines plays role in several processes such as alteration of gene expression especially genes involved in carcinogen metabolism leading to form the ultimate carcinogen, which enhance the effect of DNA adduct. Usually DNA damage lead to either DNA-mismatched repairing mechanisms, or if the damage is beyond repaired to cell death through apoptosis, then the mutated cells are permitted to survive and the progress step is going to transform to be malignant cells. Eventually, histomorphological changes occurred. The CCA usually arises from precancerous lesions in the biliary tract following the metaplasia-dysplasia-carcinoma, respectively (Yamagiwa and Tomiyama, 1986). The proposed mechanisms are summarized in Figure 11.

3.6 Treatment of Cholangiocarcinoma

CCA is a predominantly fatal cancer, which can be difficult to diagnose and to treat (Khan et al., 2005). The best treatment is resection, but most tumors are unresectable at diagnosis. Mean survival in unresectable patients is less than 6 months. Radiation has been given post-op or in unresectable cases, but usually makes a difference. Chemoradiation protocols show some promise in extending survival. Many chemotheraputic agents have been used to treat CCA without great success (Goldberg,

2004). Most frequently chemotheraputic agents used for treating CCA patients, includes 5-fluorouracil (5-FU), epirubicin, cisplatin, doxorubicin, and mitomycin C (Lee et al., 2004; Patt et al., 2001). Extension of life in patients with CCA will depend on earlier diagnosis and possible new biopharmaceutical agents (Goldberg, 2004).

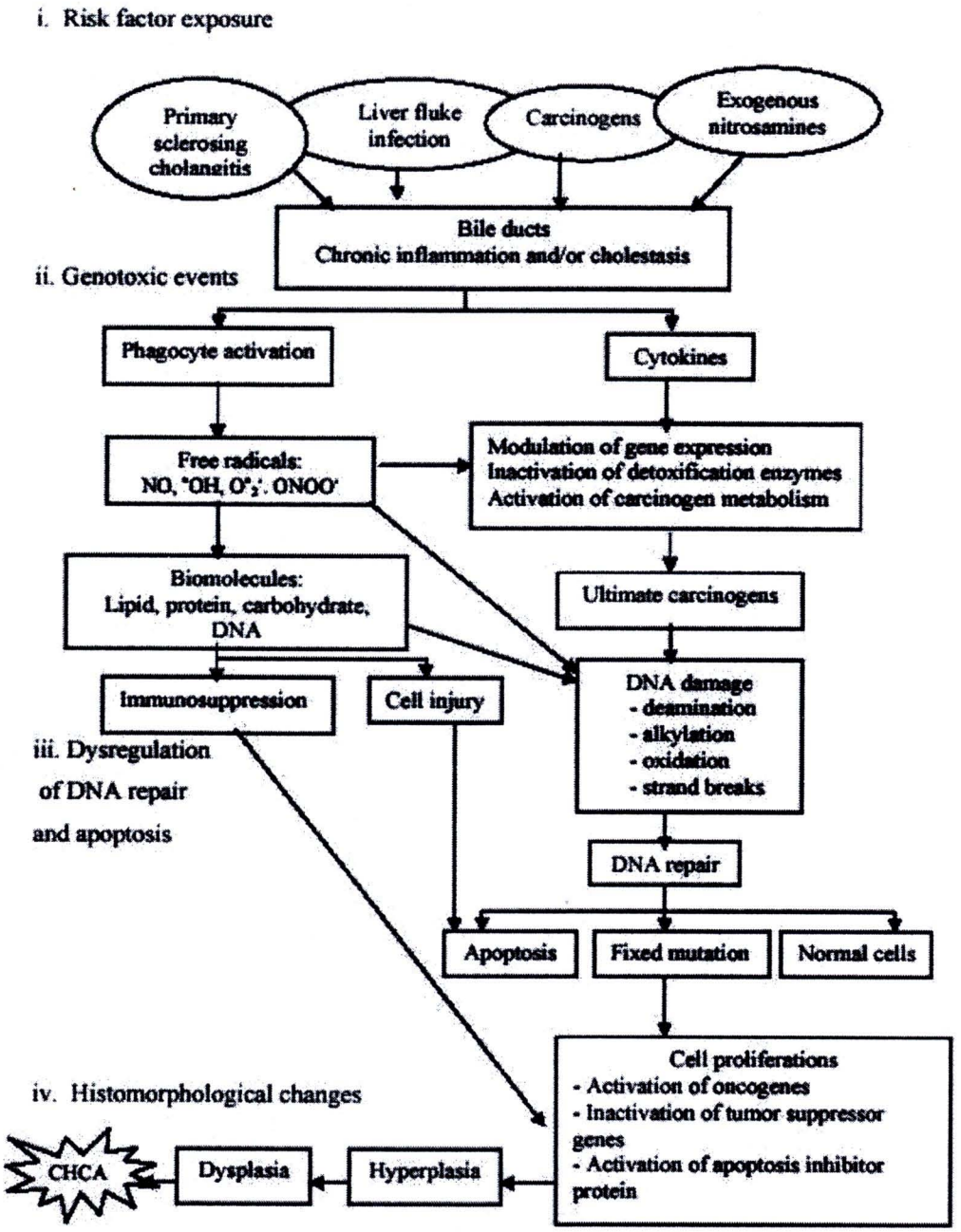

Figure 11 Mechanisms of cholangiocarcinoma (Holzinger et al., 1999; Ohshima et al., 1994).