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Invited Mini Review

Implications of NQO1 in cancer therapy

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NAD(P)H:quinone oxidoreductase (NQO1), an obligatory two-electron reductase, is a ubiquitous cytosolic enzyme that catalyzes the reduction of quinone substrates. The NQO1-mediated two-electron reduction of quinones can be either chemoprotection/detoxification or a chemotherapeutic response, depending on the target quinones. When toxic quinones are reduced by NQO1, they are conjugated with glutathione or glucuronic acid and excreted from the cells. Based on this protective effect of NQO1, the use of dietary compounds to induce the expression of NQO1 has emerged as a promising strategy for cancer prevention. On the other hand, NQO1-mediated two-electron reduction converts certain quinone compounds (such as mitomycin C, E09, RH1 and β-lapachone) to cytotoxic agents, leading to cell death. It has been known that NQO1 is expressed at high levels in numerous human cancers, including breast, colon, cervix, lung, and pancreas, as compared with normal tissues. This implies that tumors can be preferentially damaged relative to normal tissue by cytotoxic quinone drugs. Importantly, NQO1 has been shown to stabilize many proteins, including p53 and p33ING1b, by inhibiting their proteasomal degradation. This review will summarize the biological roles of NQO1 in cancer, with emphasis on recent findings and the potential of NQO1 as a therapeutic target for the cancer therapy. [BMB Reports 2015; 48(11): 609-617]

INTRODUCTION

NQO1 (NAD(P)H:quinone oxidoreductase 1) is a cytosolic flavoenzyme, which is also known as DT-diaphorase (EC 1.6.99.2) (1). NQO1 is expressed in various tissues, and its gene expression is regulated by the ARE (antioxidant response element), both in normal condition and during oxidative stress conditions (2). The NQO1 gene contains ARE in its promoter region and is regulated by the nuclear factor (erythroid- derived)-like 2 (Nrf2) (3). The NQO1 gene has been shown to be activated together with other Nrf2-induced detoxifying enzyme genes, such as GST (glutathione S-transferase) and HO-1 (heme oxygenase), in response to antioxidants, ionizing radiation, xenobiotics, heat shock, electrophiles, hypoxia, and heavy metals (1, 4).

The catalytic enzyme properties of NQO1 were first reported by Ernster and Navazio in 1958 (5). NQO1 is considered as an anticancer enzyme since it protects cells from oxidative stresses through inhibition of quinones from entering the one electron reduction to semiquinone free radicals and ROS (reactive oxygen species) (6, 7). Thus, the use of dietary compounds to induce the expression of NQO1 has emerged as a promising strategy for cancer prevention (8, 9). Recent studies have revealed that NQO1 activity is related to the risks of lung cancer (6, 7, 10, 11) or cancer of other organs (12, 13). Development of several types of human cancers has been shown to be due to NQO1 polymorphisms (14-18). Recent meta-analysis studies have shown that, in the human representative catalytic mutated NOQ1 gene (C609T) located on chromosome region 16q22, replacement of cytosine with thymidine (609C > T) express substitution of serine for proline, thereby reducing the NQO1 enzyme activity, leading to development of several types of human cancers (11, 16-21).

Although a lowered or absent NQO1 activity has been correlated with increased susceptibility for development of human cancers (11, 21), numerous studies found that NQO1 is upregulated in a number of cancers such as breast cancer, pancreatic cancer, colorectal cancer, cholangiocarcinoma, uterine cervical cancer, melanoma, and lung cancer (22, 23). In breast, colorectal and cervical cancers, the high-level expression of NQO1 was found to be associated with the late clinical stage of the disease, poor differentiation and lymph node metastasis (22, 23). Consistently, breast and cervical cancer patients with high NQO1 expression levels show lower DFS (Disease-free Survival) and 5-year OS (Overall Survival) rates, as compared to patients having lower NQO1 expression (22, 23). In addition, NQO1 activity in many cancers is significantly higher than that in adjacent normal tissues (1, 24-26).

With its unique property of transferring two-electron by using either NADH or NADPH as reducing cofactor, NQO1 catalyzes the natural and exogenous quinones and quinineimines into hydroquinones, which are toxic (27-29). Accordingly,

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there has been considerable effort to develop bioreductive anticancer drugs, such as mitomycin C, E09, RH1, β-lapachone, and 17AAG, which are activated specifically by NQO1 and, thus are preferentially toxic to cancer cells (30-38). Importantly, it has been reported that ionizing radiation (2-4 Gy) (1, 38-40), cisplatin (1), or hyperthermia (41-42°C) (41, 42) increased the NQO1 expression levels in various human and animal cancer cells, and sensitized the cells to β-lapachone, both *in vitro* and *in vivo*.

Recent emerging studies have revealed the protective roles for NQO1 regardless of its enzymatic activities (43). NQO1 structurally binds to the important tumor suppressor p53 and increases its protein stability by inhibiting proteasomal degradation (43). Furthermore, NQO1 appears to regulate the protein stability of other proteins such as p33, p73, p33ING1b, and C/EBPα (44-48).

These studies suggested that NQO1 is a multifunctional antioxidant enzyme and an exceptionally versatile cytoprotector, which contributes to a dual function in tumorigenic progression. This review will describe the biological significances of NQO1 in cancer, with emphasis on recent findings, and the potential of NQO1 as a therapeutic target for cancer therapy.

BIOCHEMICAL PROPERTIES OF NQO1 AND CANCER PREVENTION

One way to prevent cancer development is suppression of the carcinogenic metabolic activation and preventing the production of ultimate carcinogens (49). Recent studies showed that induction of phase II enzymes, such as GST, HO-1, and NQO1, correlates with inhibition against chemical-mediated tumorigenesis in animal models, during the promotion as well as initiation stages (8, 49). Among phase II enzymes, NQO1 has been most extensively studied for its effect in preventing carcinogenesis (8, 50). The multiple and general biochemical roles of NQO1 in the protection against the promotion and initiation of cancer, can be summarized into the following four categories (Fig. 1): (i) detoxification of quinone substrates by two-electron reduction; (ii) scavenging of SOD (superoxide anion radicals); (iii) maintenance of the endogenous antioxidants such as ubiquinone and α-tocopherol; and (iv) stabilization of the suppressors p53/p73/p33 proteins.

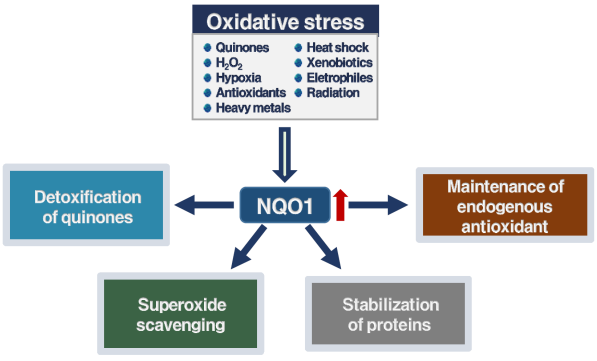

Fig. 1. The biochemical multiple and general roles of NQO1 in protection against the development of cancer.

Detoxification of quinones by NQO1

In various species including rat, zebrafish, mouse, and human, NQO1 is well known as a homodimeric flavoprotein (2, 51, 52). The best-described and widely accepted function of NQO1 is, as its name suggests, the reduction of quinones (2). Endogenous and environmental quinones are highly reactive molecules that can induce cancers and neurodegenerative diseases (53). NQO1 catalyzes the obligatory single-step two-electron reduction of the quinones to hydroquinones. Then the hydroquinones can undergo conjugation reactions (e.g., glucuronidation). Consequently, they are readily excreted from the body (2, 50). The two-electron reduction activity catalyzed by NQO1 is of benefit to the cell as it prevents generation of free radicals by redox cycle (54). Thus, the detoxification of redox-cycling quinones by NQO1 protects the cells from oxidative stresses and prevents carcinogenesis (55, 49). For example, NQO1-mediated reduction of menadione produces its formation of stable hydroquinone to be readily conjugated and excreted from the body (53). This NQO1-mediated two-electron reduction of quinones inhibits the production of unwanted one-electron reduction of them by other enzymes such as cytochrome P450 (50, 56, 57). The oxidoreductase functions have been proposed to involve a hydride transfect between the NADH and FAD cofactors and from FADH2 to the quinone substrate (50, 53). NQO1 can reduce a very broad range of substrates, including quinones, dichlorophenolindolphenol, quinone-imines, methylene blue, glutathionyl-substituted naphthoquinones, and also azo and nitro compounds (53, 56). In addition to the two-electron reduction, NQO1 is also capable of performing four-electron reduction of nitro compounds and azo dyes (58). The ability of NQO1 to reduce the toxicity and carcinogenicity of various quinones has been reviewed comprehensively elsewhere (59, 60).

NQO1 as a scavenger of superoxide anion radicals

Recent studies demonstrated that NQO1 directly scavenges superoxides in an NAD(P)H-dependent manner (27, 50). This protective effect may be significant for certain tissues, such as vasculature and myocardium, in which NQO1 is highly expressed (27, 61, 62). In cardiovascular tissues, the high NQO1 enzyme activity compensates for the lows of cardiovascular superoxide dismutase expression in the detoxification reaction of superoxide anion radicals, which are produced by various sources, including xanthine oxidase, NAD(P)H oxidases, mitochondria, and uncoupled NOSs (nitric oxide synthases) in cardiovascular tissues (61).

NQO1 as an antioxidant enzyme

There has been known that NQO1 maintains certain endogenous antioxidants in their reduced and active forms (50). Oxidation of α-tocopherolquinone, which is produced by vitamin E (α-tocopherol), has antioxidant properties following reduction to α-tocopherolhydroquinone (53). NQO1 catalyzes the two-electron reduction of α-tocopherolquinone to its hydroquinone form, which then protects against lipid peroxidation of the membranes (61). Furthermore, NQO1 catalyzes the reduction of ubiquinone analogs (coenzyme Q) to their ubiquinol forms in liposomes (53). However, the *in vivo* role of the above two-electron reduction reactions remains to be elucidated.

NQO1 as a protein stabilizer

The tumor suppressor p53, which is one of critical transcription factors related to suppression of tumourigenesis, induces either growth arrest or apoptosis, in response to stresses such as DNA damage (43). The p53 protein is regulated via modification and interactions that affect its half-life (44). Under normal conditions, p53 protein is rapidly degraded due to its interaction with Mdm-2 that induces ubiquitination and proteasomal degradation (44). Pro-apoptotic stresses disrupt this interaction between the p53 and Mdm-2 proteins, allowing the p53 to accumulate (63). NQO1 has been shown to stabilize the tumor suppressor p53 protein (43). When NQO1 expression is upregulated in cancer cells under conditions of stress, NQO1 stabilizes p53 by inhibiting its proteasomal degradation. This effect was reversed by the potent NQO1 inhibitor dicoumarol, as well as other inhibitors that compete with NAD(P)H (43). This effect of dicoumarol suggests that it affects a structural change in NQO1, which inhibits interaction between the NQO1 and p53 proteins (45, 46). Although the precise mechanism by which NQO1 activity stabilizes p53 is poorly understood, the NQO1-mediated stabilization of p53 represents a unique additional mechanism by which NQO1 may protect against carcinogenesis. In addition, it has been reported that the degradation of tumor suppressor proteins p73 and p33 is regulated by ubiquitination. Recently, it has been shown that NQO1 can also inhibit p73 and p33 degradation in the presence of NAD(P)H, and protects them from 20S proteasomal degradation (44, 63). Furthermore, NQO1 appears to regulate the degradative fate of other proteins, such as p33ING1b and C/EBPα (47, 48). These finding suggest that NQO1 plays an important role as a gatekeeper, in regulating the proteasomal degradation of specific proteins.

REGULATION OF NQO1 GENE AND CANCER PREVENTION

The use of dietary compounds or synthetic chemicals to decrease the incidence of cancer has been established half a century ago (8). Numerous studies demonstrated that chemopreventive agents are Nrf2 inducers (8). Thus, the use of chemopreventive agents to induce the Nrf2/KEAP1/ARE signaling pathway, leading to the elevation of the expression of NQO1 gene, has emerged as a promising strategy for cancer prevention (8, 9). As the name of the pathway suggests, three major components are important to the transcription of the NQO1 gene: (i) ARE, DNA consensus sequences that are located in the promoter regions of the genes (53, 64); (ii) Nrf2, one of leucine zipper transcription factor, binds to the ARE, thereby signaling transcription of target genes (43); (iii) Kelch-like ECH-associated protein 1 (KEAP1) binds Nrf2 and promotes its ubiquitination and proteasomal degradation by Cul3-based ligase (8, 9).

Regulation of NQO1 gene expression

Analysis of the human, mouse and rat genes for NQO1 showed that NQO1 is located at 16q22.1 on the human chromosome, and mouse chromosome 8 (52, 53, 65, 66). The NQO1 gene consists of five introns and six exons for an approximate length of 20 kb (53). There is considerable homology between the human and rat NQO1 coding sequences (85%) (65). The first two amino acids and the first nucleotide of the third amino acid are encoded by Exon 1, while the remaining 272 amino acids are encoded by exons 2-6 (53). NQO1 is regulated by two distinct regulatory elements in the 5′ flanking region of the NQO1 gene that are the ARE, called the EpRE (electrophile response element), and the XRE (xenobiotic response element), called the AhRE, both under basal and during oxidative stress conditions (2, 53). A variety of antioxidants, H_2O_2, and tumor promoters increase ARE-mediated NQO1 expression (53, 64). Many transcription factors can recognize ARE, TMAnnRTGAYnnnGCRwww, *in vitro*, indicating that this is a composite regulatory DNA sequences (53). Because the AP-1 binding sequences, TGASTMAG, are similar to the ARE sequences, GTGACnnnGC, AP-1 and leucine zipper proteins (bZIP) including Nrf1, Nrf2 and Maf, participate in the induction of NQO1 gene (53, 67). A model of ARE-mediated regulation of hNQO1 is proposed by Wasserman and Fah1 (68). The ARE core sequence (RTGAYnnn) interacts with the bZIP transcription factors (Jun, Fos, Fra, Nrf, Maf, Raf and NF-E2) (53). It has been known that the Nrf2-KEAP1/ARE signaling pathway is the major regulator of cytoprotective responses to oxidative and electrophilic stresses (69). XRE-mediated gene expression involves the liganded aromatic hydrocarbon receptor (AHR). The XRE-mediated gene expression is increased by PAS (Per, Arnt, Sim) family of proteins (68). The AHR/Arnt dimer interacts with the DNA sequences, XRE (70). TCDD and polycyclic aromatic hydrocarbons induce NQO1 gene expression (71). However, one study 8 reported that, in mouse hepatoma cells, TCDD-induced human NQO1 was ARE- mediated and not dependent on XRE (72).

Chemoprevention by upregulating NQO1

Environmental carcinogens, including quinones, are first metabolically activated via the phase I enzymes such as cyto-

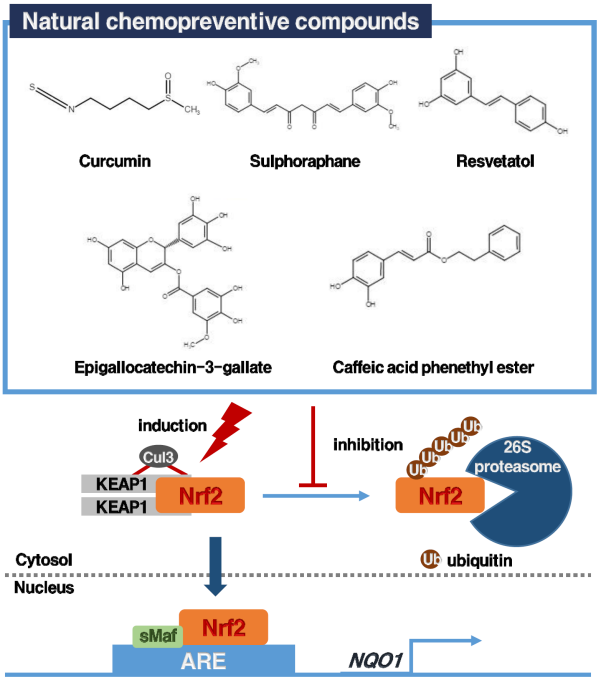

Fig. 2. Representative chemopreventive agents-induced activation of Nrf2/KEAP1/ARE signaling pathway and regulation of NQO1 gene.

chrome P450 into reactive intermediates (8). At the cellular level, there exist competing phase II enzymes (e.g. NQO1) which eliminate the reactive forms of carcinogens through biotransformation reactions, including quinone reduction, acetylation, sulfation and glutathione conjugation (8). Therefore, the use of dietary compounds or synthetic chemicals to shift the balance between phase I and II enzymes, is a promising strategy for cancer chemoprevention (73-75). The concept of chemoprevention strategy is closely correlated with Nrf2/KEAP1/ARE signaling pathway-induced expression of NQO1 (8). Many studies reported that potent Nrf2 inducers can be obtained from plants including cruciferous vegetables (sulforaphane), a wide used spice (curcumin), green tea (epigallocatechin- 3-glaate), grapes (resveratrol), conifer trees (caffeic acid phenethyl ester) and Japanese horseradish (wasabi) (8). This chemopreventive natural compounds inducing Nrf2/NQO1 signaling pathway are continuously growing, and these have been reviewed comprehensively elsewhere (9). The signaling pathway is summarized in Fig. 2.

NQO1 gene polymorphisms

It has been known that NQO1 polymorphisms increase the susceptibility for developing cancer (6, 7, 10-18). Two types of polymorphisms of the NQO1 gene have been reported in humans (43). The prominent one is a single nucleotide mutation, which is replacement of cytosine with thymidine (609C > T), of the NQO1 gene (13, 43). This mutation produces a proline to serine substitutions at position 187 of the amino acid sequence of the NQO1 (6, 7, 10-18). Another mutation NQO1*2 protein is rapidly degraded by the proteasome (76). In addition, it has been reported that the null phenotype or deficiency for NQO1 increases the susceptibility to the neoplastic and toxic effects by benzene (43, 77). A large number of studies reported that the NQO1 polymorphisms correlate with the susceptibility for developing several types of cancer. However, the results showed inconsistency because of the small sample size in the majority of studies (16). In order to overcome the problem of low statistics, a few number of meta-analyses were performed in individual studies. However, these meta-analyses considered individual cancer sites separately (16). Therefore, a global meta-analysis to investigate the role of NQO1 polymorphisms should be conducted.

THE POTENTIAL OF NQO1 FOR CANCER THERAPY

Although absent or lowered NQO1 activity has been associated with the susceptibility for developing several types of human cancers as presented above (6, 7, 10-18), the clinical significance of expression levels of NQO1 in human cancers has not been fully elucidated. In humans, NQO1 is overexpressed in a variety of solid tumors, including those of the adrenal gland, breast, colon, bladder, liver, ovary, cervix, pancreas lung, and thyroid (6, 22, 23, 78-80). In cancers, this feature has been exploited to activate anticancer drugs that are bioreductively activated by NQO1. In addition, there has been growing interest in the development of strategies to induce NQO1 activity in cancer cells for increasing the efficacy of bioreductive anticancer drugs.

Bioreductive quinone substrates for NQO1

Bioreductive anticancer drugs such as mitomycin C (MMC), β-lapachone and benzoquinone ansamycins, are activated by NQO1. MMC is a quinone containing antibiotic isolated from Streptomyces caespitosus. For more than 30 years, MMC has been used for the treatment of solid human tumors including breast, lung, pancreas and stomach (81). The mechanism of action of MMC is intracellular bioreductive activation which lead to DNA interstrand crosslinking (81). Since MMC is bioactivated by NQO1, the level of NQO1 is a good predictor of MMC sensitivity. In specifically hypoxic and acidic tumor microenvironments, other bioreductive enzyme can effectively activate MMC (82). Therefore, NQO1 expression level and NQO1 polymorphsim may not be important to determine the clinical response to MMC therapy (81). Another representative quinone, β-lapachone, is a naturally occurring ortho napthoquinone isolated from the bark of the lapachon tree (Tabeduia avellanedae) (1, 38, 83, 84). NQO1-induced activation of β-lapachone showed anti-trypanosomal, anti-fungal and anti- bacterial properties by production of hydrogen peroxide and superoxide with the simultaneous oxidation of reduced pyridine

nucleotides (85). Early studies reported that β-lapachone could inhibit topoisomerase I, thereby inhibiting the repair of DNA in mammalian cells (81). β-lapachone (ARQ 501) is processed in the Phase I and II clinical trials for the cancer therapy (81). Finally, the benzoquinone ansamycins including geldanamycin (GA) and 17-AAG are a group of quinone. GA, which is isolated from Streptomyces hygroscopicus, has anticancer properties by inhibiting RNA and DNA replication (86). It has also been reported that GA could inhibit the activity of vSrc and inhibit the expression of the cMyc (81). In addition, GA targets the heat shock protein 90 (Hsp90) by inhibiting its ATPase activity (81). GA effectively inhibits Hsp90-mediated maturation of many oncogenic proteins such as HER2, Raf-1, KIT, BCR-ABL as well as steroid hormone receptors (81). Therefore, GA and its analogs such as 17-AAG and 17-DMAG have shown anticancer effect in various human cancers. NQO1 can convert 17-AAG to the hydroquinone of 17-AAG. The hydroquinone of 17-AAG, IPI504 (Retaspimycin), was developed as a more water-soluble alternative to 17-AAG. The IPI504 actively inhibits Hsp90 and shows markedly more potency than the parent quinone (87). In recent years, several new NQO1-dependent anticancer compounds have been developed, such as 2,5-diaziridinyl-3-3[hydroxymethyl]-6-methyl-1,4-benzoquinone (RHI); 3-hydroxy-5-aziridinyl-1-methyl-2 [indol-4,7- dione]-prop-b-en-a- ol (EO9); and 3,4-dihydro-2, 2-dimethyl-2H-naphthol[1,2-b]pyran-5,6-dione (β-lapachone) (83).

Upregulation of NQO1 in cancer therapy with bioreductive anticancer drugs

Cytotoxic quinone anticancer drugs may have the advantage of preferentially damaging the cancer cells when the cancer cells when the NQO1 enzyme is upregulated or overexpressed, relative to their action on cancer cells in normal conditions (1, 38, 83, 84). Recently, it has been shown that ionizing radiation at clinically relevant doses (e.g., 2 Gy) significantly upregulates the NQO1 level in cancer cells, and sensitizes the cells to β-lapachone (38, 39). When cells expressing NQO1 are treated with a combination of ionizing radiation and β-lapachone, positive feedback regulation between ROS and ERK leads to ER stress, inducing mitochondrial translocation of cleaved Bax and JNK activation. Subsequently, the decrease of mitochondrial membrane potential leads to translocation of AIF and apoptosis (84). We have also recently reported that hyperthermia (e.g. 41-42°C) increases the enzymatic activity of NQO1 and Hsp70-mediated stabilization of NQO1, and sensitizes the cells to β-lapachone *in vitro* (41, 52, 83). Heat shock elevates NQO1 expression by cis-acting elements such as ARE and XRE. The degradation of NQO1 protein in heat-treated cancer cells was slower than in untreated cells. After heating, the Hsp70 co-localized and co-precipitated with NQO1 in cancer cells, indicating the association of these two proteins in cancer cells (83). In addition, experimental mouse tumors or human tumor xenografts could be markedly sensitized to β-lapachone treatment by heating the tumors 24 h prior to β-lapachone treatment (41, 42). Furthermore, cisplatin significantly upregulates NQO1 in cancer cells, thereby markedly increasing the sensitivity of the cancer cells to β-lapachone *in vitro* as well as *in vivo*. These data suggested that local treatment of tumors with established cancer therapies such as hyperthermia or radiotherapy, which is summar-

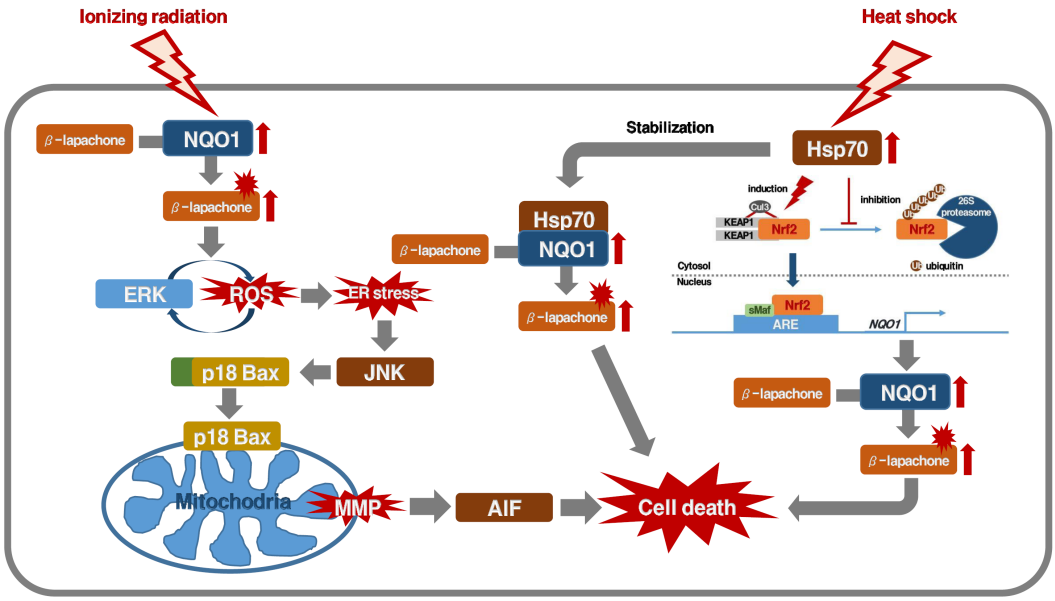

Fig. 3. Schematic model of how ionizing radiation or hyperthermia potentiates NQO1-dependent β-lapachone-induced cancer cell death.

ized in Fig. 3, may upregulate NQO1 in the tumors and selectively sensitize the cancer cells to β-lapachone. Phase I and II clinical trials are conducted in progress to determine the feasibility of using β-lapachone alone or in combination with other anticancer drugs against human solid tumors (88). In order to improve the delivery and clinical efficacy of β-lapachone to tumors are being investigated (89-91).

CONCLUSION

There has been accumulating evidence exhibiting the versatile cytoprotective role of NQO1, in particular for cancer prevention and protection from oxidative stress-related diseases. A large number of studies reported the role of NQO1 polymorphisms in susceptibility for generation of several types of cancer. Importantly, certain compounds become cytotoxic due to reduction mediated by NQO1. Interestingly, NQO1 is overexpressed in providing an opportunity to preferentially damage cancers relative to normal tissues, using bioreductive anticancer drugs. Furthermore, there has been growing interest in the development of strategies to induce NQO1 activity in cancer cells for increasing efficacy of bioreductive anticancer drugs.

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