

## Health Beneficial Effects of Food Factors Can Be Applicable to Humans?

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# Modulation of protein quality control systems by food phytochemicals

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There is compelling evidence showing that dietary phytochemicals have exhibited pronounced bioactivities in a number of experimental models. In addition, a variety of epidemiological surveys have demonstrated that frequent ingestion of vegetables and fruits, which contain abundant phytochemicals, lowers the risk of onset of some diseases. However, the action mechanisms by which dietary phytochemicals show bioactivity remain to be fully elucidated and a fundamental question is why this class of chemicals has great potential for regulating health. Meanwhile, maintenance and repair of biological proteins by molecular chaperones, such as heat shock proteins, and clearance of abnormal proteins by the ubiquitin-proteasome system and autophagy play central roles in health, some disease prevention, and longevity. Interestingly, several recent studies have revealed that phytochemicals, including curcumin (yellow pigment in turmeric), resveratrol (phytoalexin in grapes), quercetin (general flavonol in onions and others), and isothiocyanates (preferentially present in cruciferous vegetables, such as broccoli and cabbage), are remarkable regulators of protein quality control systems, suggesting that their physiological and biological functions are exerted, at least in part, through activation of such unique mechanisms. This review article highlights recent findings regarding the effects of representative phytochemicals on protein quality control systems and their possible molecular mechanisms.

**Key Words:** heat shock protein, ubiquitin-proteasome, autophagy, hormesis, phytochemical

### Phytochemicals as Plant Secondary Metabolites

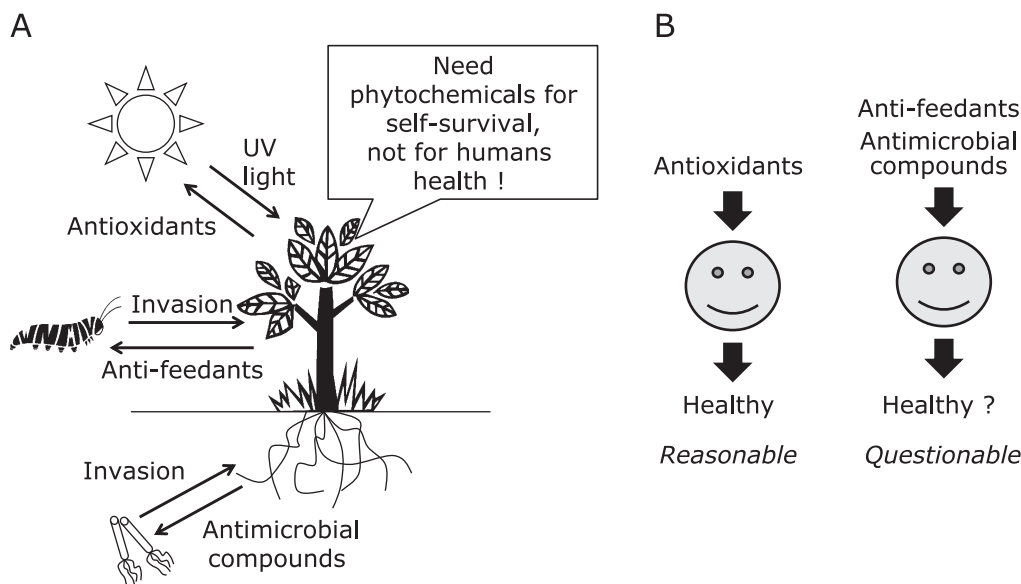
All organisms are exposed to environmental stresses, which are initiated and promoted by physical, chemical, and biological stimuli. In most cases, these stresses are actively produced to acquire biological predominance over other species and have occasionally been associated with natural selection. Plants have a critical disadvantage for survival as compared with animals, as they are unable to move to avoid biological enemies and stress stimuli, such as invading microorganisms and insects, herbivorous animals, and intense sunlight. Thus, they have developed specific biological systems that can adapt to and counteract against various stresses. For example, lignins, an integral part of the secondary cell walls of plants, serve as a physical barrier against invading organisms.<sup>(1)</sup> Also, pathogen-infected plant cells and tissues,

whose functions become irreversibly disrupted, are efficiently removed by the process of apoptotic cell death for the survival of the whole plant.<sup>(2)</sup> In addition, plant secondary metabolites, biosynthesized in both constitutive and inducible manners, function as a central group of phytochemicals that have marked potential to fight against and mitigate exogenous stresses. For example, flavonoids have notable anti-oxidant activity, which is considered to play a major role in protection against UV light-induced oxidative damage, as well as anti-fungal and anti-microbial activities (Fig. 1). Volatile terpenoids, interesting phytochemicals used as essential oils for cosmetics and perfumes, occasionally function in plants as 'infochemicals' to warn of an attack to neighboring plants for species preservation.<sup>(3)</sup> Furthermore, sulfur-containing compounds in cruciferous plants, such as isothiocyanates (ITCs), are powerful chemical weapons because of their substantial toxicity, while the precursors of ITCs, glucosinolates, are known to be hydrolyzed by chemical and physical stimuli to generate bioactive ITCs.<sup>(4)</sup>

There is also a great body of evidence showing that phytochemicals exhibit a wide array of physiological activities in humans. Ancient people had empirical knowledge that some plants and/or their extracts have great impact on health and disease regulation. For example, 'Ayurveda', recognized as a part of Hindu tradition and culture, utilizes various herbs and spices in a form of alternative medicine.<sup>(5)</sup> Similarly, 'Jamu' has a long history of at least 1300 years as a traditional medicine in Indonesia, in which plant rhizomes, leaves, barks, fruits, and others, are extracted and used for treatment of numerous diseases.<sup>(6)</sup> Moreover, it is of interest to point out that a significant portion of synthetic drugs are rooted in phytochemicals, which have been chemically modified by systematic derivatization for activity optimization. In addition, it is needless to mention that food phytochemicals exert versatile bioactivities, as demonstrated by the variety of research models reported. However, fundamental questions regarding how and why this class of chemicals exerts physiological activities remain to be fully answered. In other words, why phytochemicals, which are produced for plant self-defense, have beneficial effects in humans is quite a puzzling and intriguing question (Fig. 1).

Although mammals efficiently and actively absorb and utilize primary products, including sugars, protein, and lipids, as essential

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**Fig. 1.** Stress responses and the roles of phytochemicals. Plants are exposed to severe environmental stresses that induce them to biosynthesize secondary metabolites such as antioxidants, anti-feedants, and antibiotics, as well as others. Without those reactions, they will be killed by the stressors (A). On the other hand, there is accumulated evidence shows that phytochemicals greatly affect human health and disease prevention. Such effects of anti-oxidants are reasonable since oxidative stress plays major roles in the onset of numerous diseases in mammals. On the other hand, those of anti-feedants and antibiotics are quite puzzling, since such mechanisms do not seem to have significant associations with human physiological conditions (B).

nutrients, the bioavailability of phytochemicals is largely poor. For example, a green tea polyphenol, (–)-epigallocatechin-3-gallate (EGCg), has exhibited pronounced anti-oxidative, anti-inflammatory, and chemopreventive properties in numerous experimental systems,<sup>(7,8)</sup> while a bioavailability study showed that administration of EGCg resulted in substantial biotransformation, *e.g.*, glucuronidation, sulfation, and *O*-methylation, and its blood concentrations were limited.<sup>(9)</sup> In addition, though other polyphenolics, such as curcumin and proanthocyanidins, have been reported to show numerous bioactivities, they are poorly absorbed after administration to rodents and humans.<sup>(10)</sup> Along a similar line, ITCs, which exhibit marked chemopreventive and chemoprotective activities, potentially react with protein cysteine thiols and glutathione (GSH) to be biotransformed into their metabolites.<sup>(11)</sup> As noted below, though indispensable to exhibit bioactivity, this chemical property limits the ability of ITCs to be efficiently absorbed and circulate in the bloodstream. Taken together, results have shown that most, if not all, phytochemicals are substantially foreign chemicals to mammals, and it is not surprising that they are actively subjected to detoxification and excretion systems. Importantly, phytochemicals undesirable to animals occasionally induce stress responses, which are known to have partial associations with their biological and physiological functions. Major stress adaptation systems, known to be activated by phytochemicals, are described below.

### Adaptive Self-Defense Systems

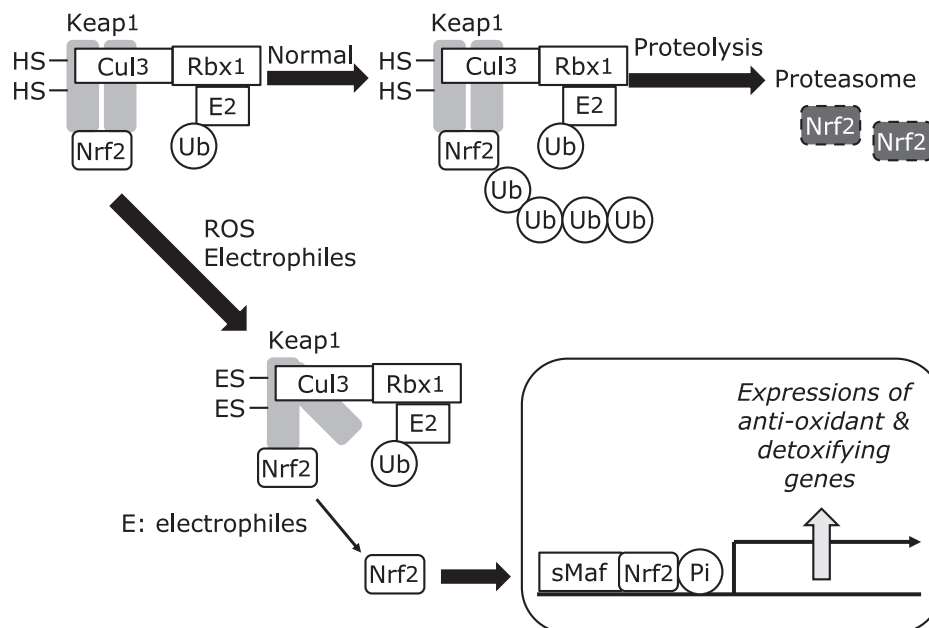
#### Anti-oxidative and xenobiotics metabolizing enzymes.

Oxidative stress plays numerous roles in pathophysiological phenomena, and thereby greatly affects health and disease onset. Biologically and chemically generated reactive oxygen species (ROS) are capable of modifying macromolecules in the human body. The Keap1/Nrf2 system adaptively functions to protect cells from oxidative and electrophilic damages by inducing a wide array of anti-oxidant enzymes (Fig. 2).<sup>(12)</sup> In a normal state, the transcription factor Nrf2 is continuously ubiquitinated by the

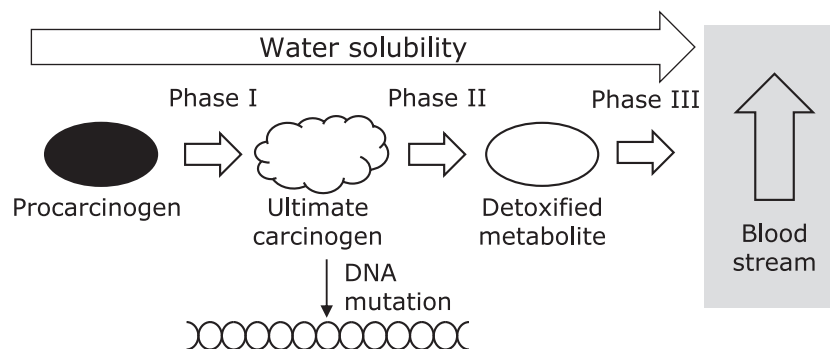
Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly subjected to degradation in proteasomes. Electrophilic chemicals and oxidative stresses oxidize the reactive cysteine residues of Keap1 in both direct and indirect manners.<sup>(12)</sup> This critical step stabilizes Nrf2, thereby inducing robust expressions of a battery of cytoprotective genes, including anti-oxidative genes, and protein quality controlling genes (molecular chaperones, ubiquitin/proteasome systems).<sup>(12)</sup> On the other hand, most foreign chemicals have a molecular hydrophobic property and are primarily modified by Phase I enzymes, such as cytochrome P450s (CYPs), which add a hydrophilic functional group to them (Fig. 3). Subsequently, Phase II enzymes, such as GSH-S-transferases (GSTs), convert Phase I enzyme-activated metabolites into water-soluble ones. Finally, Phase III transport and exclusion systems, such as P-glycoproteins and multidrug resistant proteins, transfer those conjugated metabolites into the bloodstream in an ATP-dependent manner. The above-mentioned Keap1/Nrf2 system is responsible for transcription of Phase II enzyme genes. Importantly, the ratio of Phase I and II enzyme activities is the essential determinant of the potential risk for chemical carcinogenesis (Fig. 3).<sup>(13)</sup> Though selective Phase II enzyme induction has been proposed to be beneficial for chemoprevention, potential side-effects have been recently discussed.<sup>(14)</sup>

**Heat shock proteins (HSPs).** Stress-induced denaturing of biological proteins greatly affects their conformation and critically disrupts their biological functions. A number of recent studies have indicated that several distinct protein quality control (PQC) systems play key roles in counteraction against ‘proteo-stress’. HSPs, highly conserved families of proteins ubiquitously expressed in most types of cells, allow misfolded and unfolded proteins to achieve functional conformation (Fig. 4). Thus, the expression and activity status of HSPs are considered to be critical determinants of homeostasis, as well as health and longevity. In fact, maintenance of HSPs at high levels substantially contributes to extended lifespan.<sup>(15)</sup>

HSPs are comprised of numerous family proteins, and can be divided into 2 distinct groups of constitutive and inducible iso-



**Fig. 2.** Action mechanism underlying Nrf2 activation following oxidative and electrophilic stresses. In a normal state, the transcription factor Nrf2 is continuously ubiquitinated by the Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly subjected to degradation in proteasomes. Electrophilic chemicals and oxidative stresses oxidize the reactive cysteine residues of Keap1 for reducing the E3 ligase activity. This critical step stabilizes Nrf2 and thereby induces robust expression of a battery of cytoprotective genes.

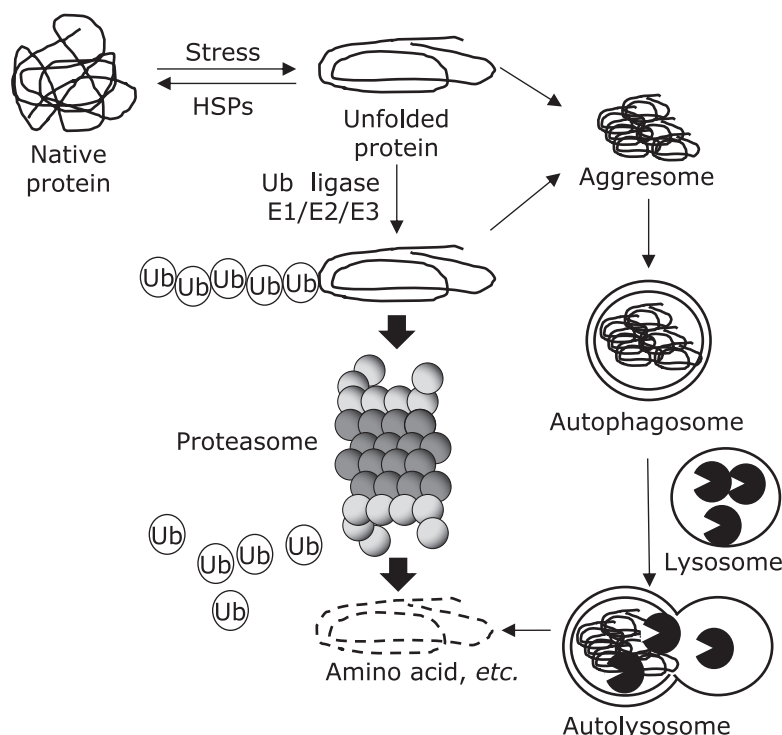


**Fig. 3.** Xenobiotics metabolism, transport, and exclusion mechanisms function through concerted activation of enzymes and proteins in Phase I, II, and III enzymes and proteins. Most environmental xenobiotics, such as procarcinogens, are biologically activated by Phase I enzymes, and the resultant metabolites are capable of mutating DNA to induce tumor initiation. Alternatively, the bioactive pro-carcinogens can be detoxified by the functions of Phase II enzymes, which provide hydrophilic groups, including  $\beta$ -glucuronides and sulfated metabolites. Those metabolites are then transported and exit from the cell into the bloodstream in an ATP-dependent manner. Generally, the water solubility of the compounds increases at each stage.

forms. Constitutive HSPs, sharing approximately 1% of cytosolic proteins, are essential for maintaining PQC under a normal state. On the other hand, physical, biological, and chemical stressors are known to up-regulate their inducible HSPs. In addition, some isoforms are actively secreted or released by cellular damage to confer stress signaling.<sup>(16)</sup> In a normal state, HSP90 $\beta$ , the major constitutive isoform, is bound to the transcription factor heat shock factor 1 (HSF1), and thereby forcing it to be biologically dormant. Heat shock and some other stimuli are capable of dissociating this heterodimer complex. Thereafter, the resultant free HSF1 forms a trimer complex and is phosphorylated at multiple sites, finally translocating into the nucleus to induce a number of HSP genes to amplify defense capacity.<sup>(15)</sup> HSP70, a major inducible isoform, protects neurons from protein aggregation and apoptosis, which may contribute to regulate Parkinson's, Alzheimer's, polyglutamine diseases, and amyotrophic lateral

sclerosis (ALS).<sup>(17)</sup> Along a similar line, overexpression of HSP-16.2, a small molecular chaperone in the nematode *Caenorhabditis elegans*, has been shown to cause a decrease in  $\beta$ -amyloid peptide toxicity.<sup>(18)</sup>

Interestingly, several recent studies have revealed that HSPs act as not only as molecular chaperones, but also have other biological functions. For example, expression of cyclooxygenase-2, a pro-inflammatory gene, was decreased in cells exposed to heat shock, which was associated with activation of HSF1, increased HSP72, and inhibition of nuclear factor  $\kappa$ B (NF $\kappa$ B), the master transcription factor for pro-inflammation processes.<sup>(19)</sup> Similarly, Lunova *et al.*<sup>(20)</sup> recently reported that over-expression of HSP72 accelerated the recovery from caerulein-induced acute pancreatitis by targeting NF $\kappa$ B. HSP27 over-expression was also reported to mitigate cytokine-induced islet apoptosis and streptozotocin-induced diabetes.<sup>(21)</sup> However, HSP up-regulation is not neces-



**Fig. 4.** Native proteins are continuously exposed to various stresses and may be denatured by unfolding. HSPs are able to repair the abnormal structures to become functional. If the stress is too severe to the extent that HSPs are not able to perform repair, those abnormal proteins are subjected to the UPS, leading to proteasome-dependent degradation. Alternatively, unfolded proteins form aggregates, which are digested by the formation and fusion of aggresomes and lysosomes. Amino acids thus produced in the UPS and by autophagy can be reused to maintain homeostasis.

sarily beneficial for human health and disease prevention, because highly expressed HSPs accelerate the growth of tumor cells on account of their drug-resistant phenotypes.<sup>(22)</sup> Therefore, HSPs are attractive targets for chemotherapeutic agents. In fact, specific HSP90 inhibitors are now considered to be promising anti-cancer drugs as a single agent or in combination with other types of agents, because HSP90 client proteins are involved in multiple oncogenic processes.<sup>(23)</sup> Collectively, it is important to keep in mind that both the benefits and risks of HSP up-regulation are essentially dependent upon the biological status of cells (normal or transformed), and thus upon the target population (healthy individuals or cancer patients).

**Ubiquitin-proteasome system.** A wide variety of organisms possess a homeostatic mechanism, called the ubiquitin-proteasome system (UPS), which degrades disused, harmful, and denatured proteins together with some constitutive ones (Fig. 4).<sup>(24)</sup> The UPS-mediated post-translational protein modification and degradation are indispensable for homeostatic phenomena, including cell cycle regulation, DNA repair, and apoptosis. It is also recognized that the UPS is the major route by which proteins are selected for temporal and spatial degradation in eukaryotic cells.<sup>(25)</sup>

The UPS cascade is executed by distinct ATP-dependent steps,<sup>(26)</sup> and requires transfer of ubiquitin from an ubiquitin-activating enzyme (E1) to a ubiquitin-conjugating enzyme (E2) and then to the target substrate protein facilitated by a ubiquitin-protein ligase (E3). This process is repetitively cycled, during which ubiquitination generally occurs through covalent attachment to ubiquitin Lys48 to form polyubiquitin chains. Polyubiquitinated substrates thus produced are then selectively transported for degradation by the 26S proteasome (Fig. 4). It is notable that a minimum of 4 ubiquitins are necessary for proteasome-dependent degradation. Interestingly, before digestion, a ubiquitin is removed from the target protein and recycled, and finally the target protein

is broken down into small peptides and amino acids.<sup>(26)</sup> Oxidative stress is known to cause protein damage and DNA mutation, both of which may induce dysfunction of the proteasome. Such events eventually lead to aberrant aggregation or incorporation of ubiquitinated proteins into hallmark structures or activation of cell death pathways.

Dysfunction of the UPS is often associated with the onset of many diseases, such as Huntington's,<sup>(27,28)</sup> Parkinson's,<sup>(29,30)</sup> Alzheimer's,<sup>(31,32)</sup> and polyglutamine diseases.<sup>(33)</sup> Also, it has recently been revealed that UPS impairment significantly affects the maintenance of cardiac function, leading to cardiac dysfunction.<sup>(34,35)</sup> Therefore, UPS-targeted drugs are anticipated to contribute to promising therapeutics for those diseases.<sup>(36)</sup> However, restoration or activation of the UPS is not definitely beneficial. For example, UPS subunits are over-expressed during the early stage of disease progression in mutant SOD1 mice, a model of ALS, suggesting that accelerated UPS functions are associated with the pathological features of ALS.<sup>(37)</sup> In addition, UPS components, especially the ubiquitin ligases MAFbx/atrogenin-1 and MuRF1, have a role to promote skeletal muscle atrophy.<sup>(38)</sup> Furthermore, drugs that inhibit the UPS have attracted attention as promising agents for chemotherapy.<sup>(39)</sup> In fact, the US Food and Drug Administration first approved the proteasome inhibitor bortezomib, as an anticancer-drug for clinical tests,<sup>(40)</sup> though the novel proteasome inhibitors are still anticipated based on reports of non-responders toward this drug.<sup>(41)</sup> In any case, the UPS appears to have both positive and negative effects on health, and its dysregulation is a hallmark of many disorders.

**Autophagy.** In 1966, Christian de Duve<sup>(42)</sup> first described the morphological process of cell self-digestion and coined the term autophagy. Autophagy is conserved from yeast to humans as a PQC process that involves recognition and turnover of damaged proteins, and is also a response mechanism to nutrient starva-

tion.<sup>(43)</sup> In mammalian cells, autophagy can be subdivided into macro-autophagy, micro-autophagy, and chaperone-mediated autophagy (CMA).<sup>(44)</sup> In macro-autophagy, a double-delimited autophagosome sequesters the cytoplasm in a large and non-specific way, and then fuses with the protease-rich acidic lysosome for protein degradation. On the other hand, micro-autophagy refers to the direct engulfment of the cytoplasm by a lysosome, while the lysosomal membrane is randomly invaginated and differentiated into the autophagic tube to enclose portions of the cytosol. CMA was discovered in 1981 and that study noted that the chaperone heat shock cognate (HSc) 70 recognizes and combines the proteins with a KFERQ or a KFERQ-like motif, then binds to the LAMP-2A, which transfers both the chaperone complex and the targeted protein into the lysosomal lumen.<sup>(45)</sup> Execution of autophagy has been shown to be tightly regulated by complex mechanisms involving diverse input signals, including nutrients, hormones, intracellular Ca<sup>2+</sup> concentrations, ATP levels, hypoxia, and importantly, accumulation of aggregated proteins.<sup>(46)</sup>

Although the molecular mechanism of autophagy has yet to be fully elucidated, Atg family proteins have initial and essential roles.<sup>(47,48)</sup> In its early stages, the Atg12-Atg5 complex and the cytoplasmic form of LC3 are recruited to a membrane particle. The isolated membrane is then reorganized into an autophagosome, and the Atg12-Atg5 complex is released. During this time, the autophagosome is matured and LC3-I is converted into its membrane-bound form, LC3-II. Thereafter, the mature autophagosome is fused with a lysosome to form an autolysosome, where the target, unnecessary cellular proteins are degraded by a cocktail of proteases (Fig. 4). Recently, several studies have revealed that the autophagic mechanism is not limited to the PQC system, but also involves clearance of dysfunctional organelles and foreign organisms, *i.e.*, mitophagy,<sup>(49)</sup> pexophagy,<sup>(50)</sup> reticulophagy,<sup>(51)</sup> nucleophagy,<sup>(52)</sup> and xenophagy,<sup>(46)</sup> which respectively refer to the selective removal of mitochondria, peroxisomes, endoplasmic reticulum, nuclei, and intruding microorganisms. Furthermore, Singh *et al.*<sup>(53)</sup> reported significant observations of lipophagy, in which autophagy involves the delivery of lipid droplets for lysosomal degradation, suggesting that an impaired autophagy could lead to the obesity- and type-2 diabetes-associated disorders.

Another important finding related to this area is that autophagy disruption may be related to inflammatory-associated disease onset. An anti-inflammatory property of autophagy is reasonable since loss or decreased autophagy may be associated with necrotic death, which can initiate an inflammatory reaction in phagocytes.

In fact, Schrijvers *et al.*<sup>(54)</sup> have proposed a hypothesis stating that autophagy plays a preventive role in the development of atherosclerosis by degrading the disused intracellular components. Similarly, autophagic, lysosomal and mitochondrial dysfunctions have been proposed to be key processes in the pathogenesis of pancreatitis.<sup>(55)</sup> In addition, defective autophagy was shown to result in an impaired anti-bacterial response, contributing to the onset of inflammatory bowel disease, such as Crohn's disease.<sup>(56)</sup> Similar to the cases of HSPs and the UPS, autophagy activation is a promotional factor for tumor growth based on efficacy for increased survival.<sup>(57)</sup> Likewise, Ryter *et al.*<sup>(58)</sup> reported that either impaired or accelerated autophagic activity affects pulmonary vascular disease. Collectively, autophagy is also recognized as a double-edged sword in terms of its influences on our health and disease regulation.

## Phytochemicals that Regulate Protein Quality Control Systems

The effects of phytochemicals on anti-oxidant and xenobiotics metabolizing enzymes have been extensively discussed elsewhere,<sup>(59–62)</sup> thus this review will highlight findings related to PQC systems, *i.e.*, HSR, the UPS and autophagy.

**Curcumin.** Curcumin (Fig. 5), the major yellow pigment in turmeric (*Curcuma longa*, Zingiberaceae), has attracted great attention from scientists in various fields (pharmacology, food, medicinal and nutritional chemistry, etc.). Notably, this phytochemical has been reported to show low or no toxicity. In fact, Chainani-Wu<sup>(63)</sup> reported that a Phase I human trial with 25 subjects using up to 8000 mg of curcumin per day for 3 months resulted in no toxicity, while 5 other human trials that utilized from 1125–2500 mg of curcumin per day have also found it to be safe. On the other hand, there is a large body of evidence showing that curcumin has versatile biological and physiological activities, such as anti-inflammatory, anti-neurodegenerative, anti-Alzheimer's disease, anti-obesity, anti-oxidative, anti-cancer, and anti-HIV activities.<sup>(64)</sup> Interestingly, recent molecular interaction studies using surface plasmon resonance, Forster type fluorescence resonance energy transfer, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and others, identified its multiple targets that confer its bioactivities.<sup>(65)</sup> At present, the binding proteins of curcumin are known to include cell survival proteins, protein kinases, protein reductases, histone acetyltransferase, histone deacetylase, glyoxalase I, xanthine oxidase, proteasome, HIV1 integrase, HIV1 protease, sarco (endo) plasmic

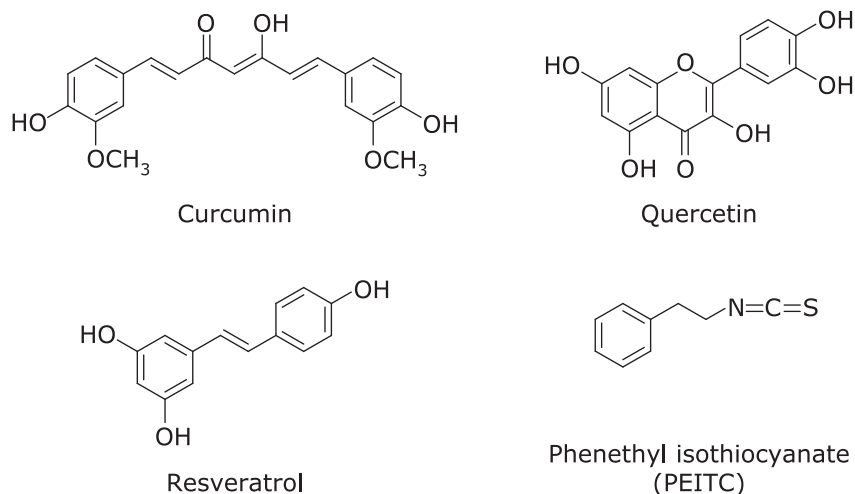


Fig. 5. Chemical structures of curcumin, resveratrol, quercetin, and PEITC.

reticulum Ca<sup>2+</sup> ATPase, DNA methyltransferases 1, FtsZ protofilaments, carrier proteins, and others.<sup>(65)</sup> It is not surprising to recognize that this agent has such a variety of targets since it is a small molecule with a simple chemical structure. It is noted that curcumin possesses the  $\alpha$ ,  $\beta$ -unsaturated 1,3-diketone moiety, which is highly susceptible to nucleophilic addition by a cysteine thiol group.<sup>(66)</sup>

In 1996, the effects of curcumin on the PQC system were demonstrated by Chen *et al.*<sup>(67)</sup> who reported that it induced the expression of the HSP70 gene in COLO205 human colon adenocarcinoma cells possibly through initial depletion of intracellular Ca<sup>2+</sup>, followed by the suppression of p53 gene function. Thereafter, curcumin was shown to prolong the stress-induced activation of HSF1 in cultured cells.<sup>(68)</sup> Interestingly, the agent also protected HK-2 human proximal tubule cells from Shiga toxin-induced cell death, possibly via up-regulation of HSP70,<sup>(69)</sup> while oral administration to SD rats with hepatic warm ischemia/reperfusion injury exhibited pronounced protective effects, which may have been due to up-regulation of self-defensive proteins, including HSP70, in the livers.<sup>(70)</sup> How curcumin induces HSR remains to be fully demonstrated. However, it has been suggested to bind HSP90 at the N-terminal domain for inhibition ( $K_d = 6.7$  nM), leading to disruption of its biochemical interaction with HSc70.<sup>(71)</sup> On the other hand, one of the early studies on the effects of curcumin on the UPS was reported by Jana *et al.*<sup>(72)</sup> who showed that exposure of the mouse neuro 2a cells to curcumin caused a dose-dependent decrease in proteasome activity and an increase in ubiquitinated proteins for apoptosis. That group also reported that curcumin increased the polyglutamine-expanded mutant huntingtin aggregation and mutant huntingtin-dependent cell death, which were accompanied with truncation of the UPS.<sup>(73)</sup>

Moreover, Milacic *et al.*<sup>(74)</sup> showed that curcumin potentially inhibited the chymotrypsin-like activities of the 20S and 26S proteasomes, and also decreased tumor growth in mice, which was associated with proteasome inhibition in tumor tissues. In contrast, some reports have noted that curcumin is a UPS inducer to promote the degradation of some oncogenic and angiogenic proteins. Chadalapaka *et al.*<sup>(75)</sup> reported that curcumin down-regulated Sp1, Sp3, and Sp4 in bladder cancer cells in a proteasome-dependent manner, and thereby degraded survivin, NF $\kappa$ B, bcl-2, and cyclin D1, all of which are related to cancer cell growth and metastasis. This phytochemical has also been shown to degrade inducible nitric oxide synthase protein of macrophages in a UPS-dependent manner,<sup>(76)</sup> indicating that it targets the enzyme at several different stages, including transcription<sup>(77,78)</sup> and post-translation.<sup>(76)</sup> Furthermore, scavenger receptors, which play major roles in oxidized low-density lipoprotein-induced cholesterol accumulation in macrophages, were degraded by curcumin in a UPS-dependent manner,<sup>(79)</sup> which may partially explain its anti-atherogenic mechanisms.<sup>(79)</sup> It is worth noting that the effects of curcumin on the UPS are coordinated with the principle of hormesis, which is characterized by an inverted U-shape dose-response.<sup>(80)</sup> This notion is supported by the study of Ali *et al.*<sup>(81)</sup> who showed that curcumin treatment (up to 1  $\mu$ M for 24 h) increased proteasome activity in keratinocytes, but significantly decreased that at a concentration of 10  $\mu$ M.

On the other hand, several recent studies have found that curcumin is a unique inducer of autophagy. For example, in 2007 Aoki *et al.*<sup>(82)</sup> reported that curcumin significantly inhibited tumor growth in a subcutaneous xenograft model of U87-MG cells and induced autophagy possibly by inhibiting the Akt/mTOR/p70S6K and activated ERK1/2 pathways. Lee *et al.*<sup>(83)</sup> also reported that curcumin induced ROS production for autophagic activation and concomitant cell death in HCT116 colon cancer cells, and a similar mechanism was shown for decreased survival of oral squamous cell carcinoma.<sup>(84)</sup> Furthermore, curcumin was found to protect from oxidative stress-induced damage in human endothelial cells via autophagy, which was executed by cytoplasmic

localization and acetylation of FOXO1 for Atg7 activation.<sup>(85)</sup> These findings clearly indicate that curcumin is a pronounced naturally occurring autophagy inducer.

**Resveratrol.** One of the earliest studies on the biological functions of resveratrol (Fig. 5), a stilbene-type polyphenol, was published by Kimura *et al.*<sup>(86)</sup> in 1985, who reported its inhibitory effects on arachidonate metabolism in leukocytes. More strikingly, Jang *et al.*<sup>(87)</sup> in 1997 reported marked chemopreventive effects of resveratrol, which inhibited the 3 distinct stages of tumor initiation, promotion, and progression. Another important issue regarding resveratrol is its possible involvement in the French Paradox, a term coined to describe the observation that the French individuals have a very low incidence of cardiovascular disease, despite a diet high in saturated fat.<sup>(88)</sup> Furthermore, in 2003, Howitz and colleagues identified resveratrol as a potent SIRT1 activator that is capable of mimicking the effects of calorie restriction,<sup>(89,90)</sup> a property considered to have connections with longevity as suggested in experimental models using yeast,<sup>(89)</sup> worms,<sup>(91)</sup> flies,<sup>(92)</sup> and fish.<sup>(93)</sup> However, recent findings presented by Burnett *et al.*<sup>(94)</sup> suggest that SIRT1 may not increase longevity in worms and flies, and currently the exact role of resveratrol and SIRT1 in longevity remain under debate.

On the other hand, the effects of resveratrol on PQC systems have already been published by a number of investigators. For example, it suppressed proliferation of human aortic smooth muscle cells, which was accompanied by a dose-dependent increase in the expression of HSP27.<sup>(95)</sup> Also, resveratrol at relatively low concentrations (50–100  $\mu$ M) was able to increase HSP70 levels and induced apoptosis of DU-145 prostate carcinoma cells, while the HSP70 level was similar to that of the control value at a high concentration (200  $\mu$ M), again showing an inverted U-shape dose-response curve by this phytochemicals.<sup>(96)</sup> Interestingly, resveratrol increased the expression of HSP70 in established cell lines and human peripheral lymphocytes, and thereby conferred thermo-resistance.<sup>(97)</sup> Furthermore, several studies have confirmed that resveratrol is an HSR inducer *in vivo*. For example, a significant induction of HSP70 was observed in the contralateral cortex of resveratrol-pretreated rats following 4 h of right middle cerebral artery occlusion.<sup>(98)</sup> In quail, dietary resveratrol ameliorated decreased food intake, egg production, and hepatic antioxidant enzymes and HSPs expression caused by heat shock (34°C for 8 h/day for 12 weeks) as compared with the control (22°C for 24 h/day).<sup>(99)</sup> Chronic intraperitoneal injection of resveratrol also increased both HSP25 and HSP70, and delayed the onset of ALS with extended survival rate in the mutated SOD over-expressing mice.<sup>(100)</sup> In contrast, however, Chakraborty *et al.*<sup>(101)</sup> found that resveratrol treatment caused HSP70 suppression at both the mRNA and protein levels in K562 chronic myelogenous leukemia, which was correlated with a diminished transcriptional activity of HSF1. Thereafter, this effect was suggested to be mediated by inhibition and activation of Akt and ERK1/2, respectively.<sup>(102)</sup> Moreover, oral feeding of resveratrol resulted in significant down-regulation of HSP70 in rat colons<sup>(103)</sup> and livers.<sup>(104)</sup> Ravagnan *et al.*<sup>(105)</sup> reported an interesting finding that resveratrol increased HSP70 expression in non-stressed human keratinocytes, but suppressed it under a heat shock condition. Taken together, the effects of resveratrol on HSP expression substantially depend on the experimental system employed (cell type, with or without heat shock, etc.).

Using proteasome subunit  $\beta$ 5-silenced cells, Marambaud *et al.*<sup>(106)</sup> showed that resveratrol has a proteasome-dependent anti-amyloidogenic activity. In addition, this agent inhibited chymotrypsin-like, trypsin-like, and post-acidic (post-glutamase) proteasome sites in RAW macrophages, and attenuated LPS-induced expressions of pro-inflammatory genes, though a mechanistic link remains to be shown.<sup>(107)</sup> On the other hand, Opipari *et al.*<sup>(108)</sup> first suggested that resveratrol is a unique autophagy inducer, as shown in a study of ovarian cancer cells. Although the molecular mecha-

nism underlying resveratrol-induced autophagy remains to be fully elucidated, Scarlatti and colleagues have suggested that it is mediated by the non-canonical Beclin 1-independent pathway.<sup>(109)</sup> Thereafter, Hsu *et al.*<sup>(110)</sup> revealed the involvement of cathepsin L in resveratrol-induced autophagy and apoptosis in cervical cancer cells. Interestingly, resveratrol at lower concentrations (0.1 and 1  $\mu$ M in H9c2 cardiac myoblast cells and 2.5 mg/kg/day in rats) induced cardiac autophagy after hypoxia-reoxygenation or ischemia-reperfusion whereas it was attenuated at higher doses.<sup>(111)</sup> Meanwhile, mechanistic findings were used to propose that the mTOR-Rictor survival pathway is important for resveratrol-induced autophagy.<sup>(111)</sup> In contrast, Yamamoto *et al.*<sup>(112)</sup> found that resveratrol-induced autophagy may be positively regulated by the p38 and ERK1/2 pathways, but not the Akt/mTOR pathway in U373 glioma cells. Furthermore, resveratrol triggered autophagic cell death in chronic myelogenous leukemia cells, by both AMPK activation and JNK-mediated p62/SQSTM1 expression.<sup>(113)</sup> Similarly, the AMPK-SIRT1-autophagy pathway has recently been shown to play an important role in the neuroprotection provided by resveratrol in cellular models of Parkinson's disease.<sup>(114)</sup> Also, resveratrol was noted to trigger autophagic cell death via the increased expression of Atg5, Atg7, Atg9, and Atg12 proteins in Huh-7 human hepatoma cells.<sup>(115)</sup> However, resveratrol may promote noncanonical autophagic degradation downstream of the phosphatidylinositol 3-phosphate-WIP1-Atg7-Atg5 pathway, by engaging a distinct subset of LC3-II.<sup>(116)</sup> Treatment with resveratrol protected against neurotoxicity caused by prion protein peptides PrP (106–126), possibly through activation of autophagic signaling.<sup>(117)</sup> In addition, Lv and Zhou<sup>(118)</sup> reported that resveratrol protected H<sub>2</sub>O<sub>2</sub>-treated H9c2 embryonic rat heart-derived cells by up-regulating autophagy via the p38 MAPK pathway. Lin and colleagues,<sup>(119)</sup> who established a rat model of cholestasis by bile duct ligation, also showed that the agent suppressed cholestatic liver injury through anti-apoptotic effects, which were accompanied with mitochondrial biogenesis and autophagy induction. In contrast, Xu *et al.*<sup>(120)</sup> found that resveratrol suppressed autophagy induced by the antibiotic doxorubicin, which is widely used in cancer chemotherapy, and thereby protected against cardiotoxicity associated with this drug.

Importantly, Pietrocola *et al.*<sup>(121)</sup> have explored active components in red wine other than resveratrol because its concentration in red wine is far too low to account alone account for the French paradox. They selected phenolic compounds found in red wine, including anthocyanins (oenin), stilbenoids (piceatannol), monophenols (caffeic acid, gallic acid), glucosides (delphinidin, kuronamin, peonidin) and flavonoids (catechin, epicatechin, quercetin, myricetin), and found that all of those components were capable of stimulating autophagy, though with various potencies.<sup>(121)</sup> In spite of the complex and occasionally contradictory mechanisms of action, modulation of autophagy by resveratrol is now attracting the attentions of many researchers.

**Quercetin.** Quercetin (Fig. 5) is a flavonol found widely throughout the plant kingdom. In addition to its pronounced antioxidant activity, this phytochemical has been reported to exhibit versatile biological activities, as shown in numerous studies.<sup>(122)</sup> An early study by Hosokawa *et al.*<sup>(123)</sup> reported that quercetin and several other flavonoids suppressed heat shock-induced HSP90, HSP70, HSP47, and HSP27 expressions in HeLa and COLO320DM cells. Regarding the mechanism of action, that group used a promoter assay to suggest that quercetin may interact with HSF1 and thereby attenuate HSP expression.<sup>(124)</sup> In addition, quercetin down-regulated the expressions of both HSP40 and HSP70 in an HCV cell culture system, which may have been related to reduced infectious viral particle production in an HCV cell culture system.<sup>(125)</sup> Meanwhile, the chemopreventive activities of quercetin have also attracted attention, though its mechanism of action is still controversial.<sup>(122)</sup> Zanini *et al.*<sup>(126)</sup> reported that quercetin inhibited the expression of multiple HSPs in neuroblastoma cells,

and caused higher sensitization of doxorubicin, and suggested a combination anti-cancer therapy that included this flavonoid. Also, quercetin was found to selectively induce apoptosis of prostate cancer cells by down-regulating the expression of HSP90.<sup>(127)</sup> On the other hand, the agent was shown to promote ubiquitination for down-regulating Her-2/neu protein, which is associated with a poor prognosis in breast cancer.<sup>(128)</sup> Similarly, treatment of several malignant glioma cells with quercetin led to proteasomal degradation of survivin, which resulted in activation of death receptor-mediated apoptosis.<sup>(129)</sup> In contrast, quercetin down-regulated myeloid cell leukaemia-1 protein, being associated with apoptotic resistance in chronic lymphocytic leukemia, by affecting both mRNA stability and the proteasome-dependent protein degradation.<sup>(130)</sup> Furthermore, quercetin induced autophagic processes and thereby reduced the half-life of oncogenic Ras protein, implying a novel chemopreventive mechanism associated with this flavonoid.<sup>(131)</sup> Also, it was suggested to induce apoptosis in gastric cancer cells by modulating Akt-mTOR signaling and hypoxia-induced factor-1 $\alpha$  signaling, and thereby exhibited autophagy in a xenograft model.<sup>(132)</sup> Meanwhile, competitive crosstalk between the UPS and autophagy has recently been reported by several independent groups.<sup>(133)</sup> For example, when quercetin inhibits proteasomal activity, polyubiquitinated protein aggregates were accumulated and autophagy was increased via marked reduction in the phosphorylation of the mTOR substrates.<sup>(134)</sup>

**ITCs.** ITCs are a family of compounds derived almost exclusively from plants, though marine sponges and fungi have also been reported to produce a few ITCs.<sup>(135)</sup> They are synthesized and stored as forms of glucosinolates in plants, and are generated by myrosinase when exposed to various stresses such as invasion by insects. In addition, when orally ingested glucosinolates may be hydrolyzed in the intestinal tract to produce ITCs, as the microflora possess a myrosinase-like activity.<sup>(136)</sup> Examples of popular crucifers that are particularly rich in certain ITCs include mustard and horseradish (allyl ITC, AITC), watercress (phenethyl ITC, PEITC; Fig. 5), and broccoli (sulforaphane).<sup>(137)</sup>

As noted above, xenobiotics metabolizing systems comprise Phase I, II, and III stages, which protect cells from a wide variety of endogenous toxins and xenobiotics, including environmental pro-carcinogens in a concerted manner.<sup>(138)</sup> ITCs are selective phase II enzyme inducers with marked activities for cancer prevention and chemoprotection.<sup>(139)</sup> For example, sulforaphane was suggested to prevent carcinogenesis in multiple organs, such as the breast,<sup>(140,141)</sup> colon,<sup>(142,143)</sup> and liver<sup>(144,145)</sup> by possibly by up-regulating these enzymes. ITCs are also chemically potent electrophiles and thus react with the thiol group of Keap1, which in turn activates Nrf2. In fact, there is a large body of evidence showing that ITCs markedly activate the Keap1/Nrf2 system for inducing numerous self-defense molecules, such as anti-oxidant and Phase II enzymes.<sup>(146)</sup> Interestingly, a cohort study of middle-aged and older Chinese individuals in Singapore found that cruciferous vegetables contain GST inducers, which were suggested to be ITCs.<sup>(147)</sup>

In 2006, Hu and colleagues<sup>(148)</sup> found that gene expression profiling of sulforaphane-treated *nrf2* wild and deficient mice resulted in identification of HSPs, ubiquitin/26S proteasome subunits, and lipid metabolism genes, all of which were up-regulated by this phytochemical. Similarly, using a gene microarray technique, Moon and colleagues<sup>(149)</sup> showed that PEITC up-regulated HSP27 in MCF-7 human breast cancer cells. In addition, enzymatic conversion of the precursor sinigrin to AITC led to marked increase of HSP70 expression in *C. elegans*.<sup>(150)</sup> In contrast, sulforaphane down-regulated the expressions of HSP70, HSP90 and HSF1 for inducing apoptosis in breast cancer cells.<sup>(151)</sup> Li *et al.*<sup>(152)</sup> also reported that sulforaphane induced the degradation of HSP90 client proteins and blocked the interaction of HSP90 with its cochaperone p50 (Cdc37) in pancreatic cancer

cells for apoptosis. Also, protein thiol modifications by ITCs are a critical step to exhibit their biological activities, and thiolated 6-methylsulfinylhexyl ITC (MSITC) was shown to target HSP90 $\beta$  to activate HSF1-dependent HSR,<sup>(153)</sup> suggesting that biochemically modified ITCs still possess biochemical activities.

Sulforaphane caused cell cycle arrest in the G<sub>2</sub>/M phase in PC-3 human prostate cancer cells, which was accompanied with proteasome-dependent Cdc25C protein degradation,<sup>(154)</sup> with similar findings were seen for PEITC<sup>(155)</sup> and benzyl ITC (BITC).<sup>(156)</sup> Moreover, PEITC degraded  $\alpha$ - and  $\beta$ -tubulin proteins in human prostatic carcinoma cell lines in a proteasome-dependent manner,<sup>(157)</sup> with similar results found in A549 lung cancer cells.<sup>(158)</sup> Furthermore, both BITC and PEITC inhibited the growth of multiple myeloma cells by inhibiting the 26S and 20S proteasomes, presumably through direct binding,<sup>(159)</sup> and that inhibition was suggested to be unrelated to either ROS generation or ITC-induced protein aggregation.<sup>(159)</sup> Proteomics studies have revealed that the molecular targets of ITCs comprise at least 30 proteins, including proteasome subunits of both the 20S catalytic and 19S regulatory complexes.<sup>(160)</sup> Herman-Antosiewicz *et al.*<sup>(161)</sup> were the first to report that treatment of PC-3 and LNCaP prostate cancer cells with sulforaphane resulted in autophagy, and proposed this to be a defense mechanism of cancer cells to protect them from sulforaphane-induced apoptosis. In support of their findings, inhibition of autophagy potentiated sulforaphane-induced apoptosis in WiDr colon cancer cells,<sup>(162)</sup> as well as anti-angiogenesis in human umbilical vein endothelial cells.<sup>(163)</sup> In addition, PEITC-induced autophagic and apoptotic death of PC-3 human prostate cancer cells, which was shown to be dependent on Atg5.<sup>(164)</sup> Furthermore, administration of PEITC decreased the incidence as well as burden of poorly differentiated tumors in the dorsolateral prostate of transgenic mice as compared with control mice possibly via induction of autophagy.<sup>(165)</sup> Interestingly, BITC induced the formation of an aggresome-like structure through covalent modifications of  $\alpha$ - and  $\beta$ -tubulin, suggesting that it has a proteo-stress effect.<sup>(166)</sup> Although the mechanism underlying ITC-induced autophagy remains to be fully elucidated, oxidative stress is proposed to have some association.<sup>(167)</sup> This notion is reasonable since ITCs have been reported to be redox regulators, that rapidly react with and thus consume cellular GSH for inducing oxidative stress.<sup>(168)</sup>

## Chemical Training Hypothesis

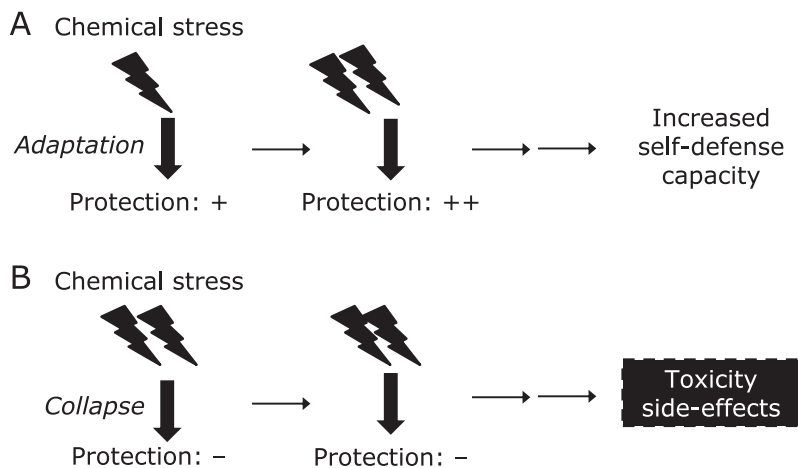
As noted above, accumulated evidence indicates that the activation of PQC systems by phytochemicals may be triggered by their specific interactions with signaling molecules, such as cell surface receptors, protein kinases, phosphatases, and transcription factors. Nonetheless, it is tempting to hypothesize that their non-specific associations with and bindings to biological proteins contribute to those proteo-static mechanisms since phytochemicals, in general, are considered to have multiple protein associations.<sup>(65,169–171)</sup> For example, ITCs may target numerous proteins including cytochrome P450s, Keap1, adenosine triphosphatase, tubulin, transient receptor potential channel, phosphatase M3/6, Cdc25c, MEKK1, epidermal growth factor receptor, PKC, GSH reductase, thioredoxin, activator protein-1, proteasome, histone deacetylase, STAT3, and mutant p53, by binding to their cysteine, lysine, and proline residues.<sup>(171)</sup> Importantly, these binding characteristics are closely associated with their electrophilic properties, allowing formation of covalent bindings to those amino acid residues. It has been shown that cellular proteins modified by 4-hydroxy-2-nonenal (HNE), a potent endogenous electrophile, were gradually removed from cells.<sup>(172)</sup> Interestingly, the recovery process was amplified by the HSR inducer, rapamycin,<sup>(172)</sup> suggesting that HNE-modified proteins are subjected to chaperones and/or the autophagy system for refolding and/or degradation.<sup>(172)</sup> Similarly, MSITC was recently shown to be bound to numerous

cellular proteins with less selectivity and activated HSF1 for inducing HSR.<sup>(153)</sup> Also, treatment of HepG2 and V79 cells with menadione, an electrophile occasionally used as a nutritional supplement, resulted in formation of non-native disulfides for protein destabilization and denaturation,<sup>(173)</sup> and hydrophobic domains were exposed on the surface of a protein, possibly via oxidative stress.<sup>(173)</sup> These findings are quite important because an extremely large number of food phytochemicals, *e.g.*, polyphenols<sup>(174)</sup> and ITCs,<sup>(175)</sup> have potential to show pro-oxidative effects. In addition, it would be reasonable to assume that hydrophobic phytochemicals might have non-specific protein interactions, leading to their alteration of functional conformation for denaturation. In accordance with the above mentioned observations, our recent results showed that electrophilic and hydrophobic phytochemicals were found to be notable HSP70 inducers in mouse hepatoma cells, and non-specific, broad protein modifications were suggested to also have a significant role.<sup>(176)</sup> Collectively, non-specific interactions of phytochemicals with cellular proteins may significantly contribute to up-regulation of PQC systems via mild proteo-stress.

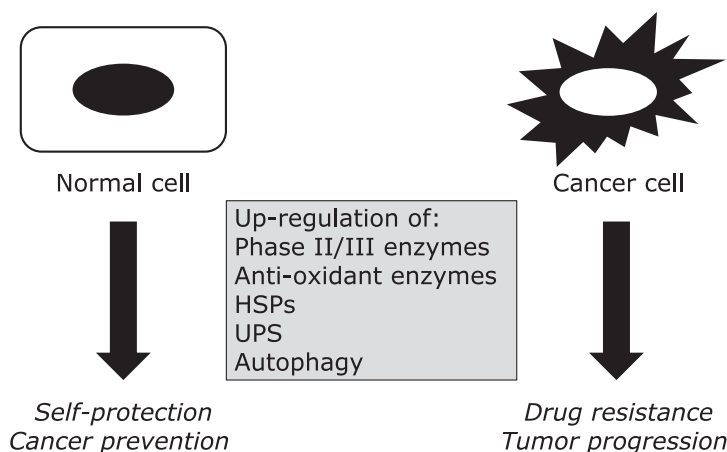
The putative, proteo-stress-activated PQC systems can be described as hormesis, an adaptive, biological mechanism that functions with low levels of exposure to toxins and other chemical stressors.<sup>(177)</sup> It is of great importance to point out that the formation of denatured proteins themselves may initiate protein repair and degradation programs to prepare for and counteract against further proteo-stress. In other words, mild proteo-stress may be the key signal to amplify this homeostatic system. Thus, repetitive exposures to appropriate doses of phytochemicals likely have positive effects to maintain protein quality. In contrast, no nutrients and harmful toxins are capable of mimicking this unique function. Also, it is essential to note that hormetic responses by chronic phytochemical ingestion involve other adaptive mechanisms, such as up-regulation of anti-oxidant and xenobiotics metabolizing enzymes.

Based on the fact that hormesis exhibits an inverted U-shape dose-response,<sup>(80)</sup> it is important to note that defense systems activated by phytochemicals would reach a plateau or even decay after being exposed to high-doses (Fig. 6), as shown in recent studies of phytochemical toxicology. For example, while green tea polyphenols at moderate or low doses protected from hepatic damage in several rodent models,<sup>(178–180)</sup> those at high-doses exhibited hepatotoxicity and nephrotoxicity.<sup>(181–183)</sup> Importantly, these harmful effects may be partially caused by collapse of self-defense machineries, including PQC systems.<sup>(182)</sup> Therefore, mild chemical stress may provide significantly beneficial effects by up-regulating adaptive responses, and excessive burdens are apparently harmful. Along the same line, chronic ingestion of phytochemicals may be referred as ‘chemical training’, which can continuously and properly stimulate adaptation systems to strengthen the defense capacity. Such putative situation resembles to the case of muscle training for physically building up the body. Concurrently, excessive chemical training by overdose dietary supplementation, for example, can be compared to overtraining. Meanwhile, hormesis is observed in other situations in addition to chemical stress-related phenomena. For example, low doses of ionizing radiation may protect from carcinogenesis by activating the DNA repair systems,<sup>(184)</sup> and could even be effective to delay the development of diabetes.<sup>(185)</sup> In addition, hot spring bathing may be good for health because it generates hydrogen sulfide, which has recently been emerged as a signaling molecule for stress adaptation.<sup>(186)</sup> Furthermore, sun exposure may affect our physical defense capacity since skin pigmentation is thought to be determined by melanocytes that produce melanin for protecting against UV radiation.<sup>(187)</sup> Thus, hormesis appears to comprise a significant portion of human daily life and have considerable effects on physiological condition.





**Fig. 6.** General scheme of chemical stress adaptation. Chemical stress has a potential to strengthen the adaptation system, allowing the host to acquire stronger resistance to harsher stresses (A). However, when the stress exceeds the defense capacity, it becomes toxic, exhibits side-effects, and is occasionally lethal (B).



**Fig. 7.** Risks and benefits of activation of self-defense systems. In normal cells, increased cell survival capacity results in amplification of stress resistance, which may be related to a healthy status and thus longevity. However, if this occurs in cancer cells, drug resistance may be increased, leading to the predominant growth of the malignant tumor cells. Thus, it should be kept in mind that the benefits of increased self-defense capacity are critically dependent on cell type and thus population.

## Conclusion and Perspectives

Modulation of PQC systems by phytochemicals is a new paradigm for elucidating the mechanisms underlying their physiological activities, since these adaptive systems are involved in numerous biochemical processes, including inflammation, carcinogenesis, and neurodegenerative diseases. As noted above, the risks and benefits of amplified PQC systems largely depend on the types of cells, tissues, and populations, in both normal individuals and cancer patients (Fig. 7). Thus, functional foods directed towards these bioactivities should be carefully investigated for their potential toxicity. Meanwhile, though food phytochemicals are known to act on specific signaling pathways, their board interactions with cellular proteins also deserve further investigation. This notion is supported by the fact that phytochemicals are biosynthesized so as to not selectively bind mammal proteins, and even are recognized as xenobiotics.

Different from synthetic drugs and natural deadly toxins, food phytochemicals can be described as ‘mild toxins’ and thus have great potential to activate adaptive self-defense systems with

lower toxicity. Phytochemicals are known to stimulate stress responses in plants via the deacetylase family of Sirtuins, which are also found in diverse eukaryotes,<sup>(188)</sup> suggesting that this class of enzymes is evolutionally maintained in various organisms. Thus, when being ingested by animals, phytochemicals may serve as useful indicators of a deteriorating environment and/or food supply.<sup>(89)</sup> The research group of Sinclair *et al.* coined the term ‘xenohormesis’ for this putative defense mechanism.<sup>(189,190)</sup> However, it can be argued that the primary molecular targets or binding proteins of most, if not all, phytochemicals are not Sirtuins, even though they modulate Sirtuin-related signaling pathways. Rather, their primary targets could be diverse proteins<sup>(11,65,169,191)</sup> via ‘dirty’ binding modes. Therefore, it is tempting to speculate that proteo-stress-triggered activation of PQC may account for significant portions of the mechanisms underlying their physiological functions. Are phytochemicals friends or foes? It can be said that they are good friends because they are weak foes, *i.e.*, this notion must be limited to situations, in which they act in a gentle manner.

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## Abbreviations

AITC	allyl ITC
ALS	amyotrophic lateral sclerosis
BITC	benzyl ITC
CMA	chaperone-mediated autophagy
CYP	cytochrome P450
EGCg	(-)-epigallocatechin-3-gallate
GSH	glutathione
GST	GSH S-transferase

H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HNE	4-hydroxy-2-nonenal
HSc	heat shock cognate
HSF	heat shock factor
HSP	heat shock protein
ITC	isothiocyanate
MSITC	6-methylsulfinylhexyl ITC
NFκB	nuclear factor κB
PEITC	phenethyl ITC
PQC	protein quality control
ROS	reactive oxygen species
SOD	superoxide dismutase
UPS	ubiquitin-proteasome system

## Conflicts of Interest

No potential conflicts of interest were disclosed.

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