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REVIEWS: CURRENT TOPICS

Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation[1] (Review)

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Abstract

Phytochemicals are potential cancer chemopreventive agents, based partly on cellular research establishing that phytochemicals inhibit the proliferation of cancer cells. To elucidate the mechanism of phytochemicals, a basic understanding is needed of what stimulates cancer cell proliferation. Cancer cells, particularly those that are highly invasive or metastatic, may require a certain level of oxidative stress to maintain a balance between undergoing either proliferation or apoptosis. They constitutively generate large but tolerable amounts of H_2O_2 that apparently function as signaling molecules in the mitogen-activated protein kinase pathway to constantly activate redox-sensitive transcription factors and responsive genes that are involved in the survival of cancer cells as well as their proliferation. With such a reliance of cancer cells on H_2O_2, it follows that if the excess H_2O_2 can be scavenged by phenolic phytochemicals having antioxidant activity, the oxidative stress-responsive genes can be suppressed and consequently cancer cell proliferation inhibited. On the other hand, phenolic phytochemicals and another group of phytochemicals known as isothiocyanates can induce the formation of H_2O_2 to achieve an intolerable level of high oxidative stress in cancer cells. As an early response, the stress genes are activated. However, when the critical threshold for cancer cells to cope with the induced oxidative stress has been reached, key cellular components such as DNA are damaged irreparably. In conjunction, genes involved in initiating cell cycle arrest and/or apoptosis are activated. Therefore, phytochemicals can either scavenge the constitutive H_2O_2 or paradoxically generate additional amounts of H_2O_2 to inhibit the proliferation of cancer cells.

Keywords: Hydrogen peroxide; Isothiocyanates; Phenolic phytochemicals; Signal transduction; Redox-sensitive genes

1. Introduction

Optimum nutrition helps reduce the risk of developing chronic diseases such as cancer. A diet that includes plenty of different fruits, vegetables, and other plant products ensures the ingestion of various phytochemicals. They include phenolics, which are very large in number and structurally diverse [1], and also glucosinolates, which are the precursors to isothiocyanates [2,3]. Although not considered nutrients in the classical sense, phenolic phytochemicals and isothiocyanates are bioactive. They have health-promoting properties, especially in terms of cancer chemopreventive activity as established by studies with animals where phenolic phytochemicals [4] and isothiocyanates [2,3] inhibited tumorigenesis induced by chemical carcinogens. The induction of enzymes involved in detoxicating the chemical carcinogens is one primary mechanism to explain the antitumorigenic effects of phenolic phytochemicals and isothiocyanates. On the other hand, these two distinct classes of phytochemicals may work as cancer chemopreventive agents by preventing a new tumor from increasing in size or limiting the proliferation of cancer cells once limited numbers of progenitor cancer cells have evolved.

At the cellular level, the molecular effects of phenolic phytochemicals and isothiocyanates in inhibiting the proliferation of cancer cells have become clearer over the past few years. To understand how these phytochemicals work within the context of this review, a basic understanding is needed of what stimulates cancer cell proliferation. Hydrogen peroxide (H_2O_2) has emerged as a pivotal molecule not

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only for cancer cell proliferation but also in determining the fate of cancer cells exposed to phenolic phytochemicals and isothiocyanates. H_2O_2 either promotes or inhibits the proliferation of cancer cells, and the phytochemicals either scavenge or generate H_2O_2. Drawing from these lines, this review will focus heavily at first on the role of H_2O_2 in overactivating signaling processes that subsequently enhance the expression of genes needed for the proliferation of cancer cells. Then, the potential of phenolic phytochemicals and isothiocynates to inhibit cancer cell proliferation by either decreasing or increasing the levels of H_2O_2 will be discussed. More specifically, the latter part of this review will conceptualize how phenolic phytochemicals and isothiocyanates modulate oxidative stress in cancer cells, thereby affecting signal transduction, activation of redox-sensitive transcription factors, and expression of specific genes that influence cell proliferation and cell death or apoptosis.

2. Constitutive oxidative stress in cancer cells

Higher amounts of reactive oxygen species (ROS), but particularly hydrogen peroxide (H_2O_2), are produced in some cancer cells. The cumulative production of H_2O_2 in human melanoma, neuroblastoma, colon carcinoma, and ovarian carcinoma cell lines was comparable to that in phorbol ester-stimulated human blood neutrophils [5]. This H_2O_2 production may have reflected initial greater generation of superoxide anion ($O_{2\cdot}$) either by the NADPH:O_2 oxidoreductase system or from the mitochondrial electron transport chain, since H_2O_2 can be formed from $O_{2\cdot}$ by the catalytic action of superoxide dismutase. To the contrary [5], the cancer cells appeared to lack NADPH:O_2 oxidoreductase (cytochrome b_{559} undetectable), and electron transport inhibitors did not prevent the increased production of H_2O_2. Furthermore, the cancer cells did not produce $O_{2\cdot}$ in stoichiometric amounts to account for their higher production of H_2O_2, which led to the conclusion that the cancer cells produced large amounts of H_2O_2 in a direct manner. However, the exact source or mechanism is unclear. The ability of cancer cells to produce large amounts of H_2O_2 is evident once they evolve as a result of transformation, which was demonstrated by a study [6] where compared to parental NIH 3T3 cells, these same cells transformed with a Ras oncogene have greater rates of ROS production [6].

Because cancer cells constitutively produce high amounts of H_2O_2, the concept of "persistent oxidative stress in cancer" originated [7], which provides a plausible explanation for some of the abnormal characteristics of cancer cells. For example, compared to their control counterparts, various human tumor tissues contain higher levels of 8-hydroxy-2′-deoxyguanosine that is indicative of greater oxidative DNA damage, as previously summarized [7] and further corroborated [8]. These findings suggest that the higher amounts of H_2O_2 increased the levels of hydroxyl radical ($^{\cdot}OH$), which can be formed from H_2O_2 in the presence of metal ions [9]. Intracellular iron (Fe^{++}) is the metal involved in the oxidative damage to DNA caused by ROS [10]. Therefore, additional gene mutations may arise in the cancer cells, further explaining their characteristic genomic instability. Other biomolecules are damaged as well. Higher levels of proteins modified by the lipid peroxidation product, 4-hydroxy-2-nonenal, and by peroxynitrite ($ONOO^-$), have been detected in carcinoma tissue compared to adenoma tissue [8].

Despite the relatively greater damage to key cellular components such as DNA, cancer cells tolerate the damage. That is, they do not undergo cell cycle arrest and/or apoptosis, which is often a consequence of the activation of p53 in response to DNA damage [11]. Instead, the cancer cells survive, and they continue to grow and proliferate as if the oxidative stress is actually conducive to their vitality. This notion suggests that the relatively large amounts of H_2O_2 being produced by cancer cells have an important role in stimulating cancer cell proliferation, rather than the H_2O_2 merely being a useless and cytotoxic product.

3. Potential for the higher constitutive H_2O_2 production in cancer cells to augment signal transduction, gene activation, and cellular proliferation

Although originally thought to be a destructive molecule specifically when transformed into hydroxyl radical [9], H_2O_2 is now widely recognized as being extremely important in signal transduction [12]. Hence, it is conceivable that the large amounts of H_2O_2 produced in some cancer cells [5] are needed to mediate signaling events that lead to activation of redox-sensitive transcription factors and responsive genes that are involved in cancer cell growth, proliferation, and survival. To illustrate this concept, Figure 1 shows how the H_2O_2 mediates signal transduction, leading eventually to the transcriptional activation of some representative genes, namely, cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMP), and cyclin B1. In terms of their relevance, COX-2 expression is higher in cells from breast cancer tissue [13], colon cancer tissue [14], and cervical cancer tissue [15] when compared to cells from normal surrounding tissue. COX-2 protein is involved in the formation of prostaglandin E_2, an eicosanoid that was recently shown to induce growth factor receptor phosphorylation and mitogenic signaling in colon cancer cells [16]. The family of MMP genes code proteins that facilitate tumor invasion and metastasis [17]. The cyclin B1 gene codes a protein that allows cell cycle progression through the G_2/M-phase checkpoint so that mitosis occurs, and is overexpressed in some types of cancer, such as squamous cell carcinoma of the esophagus [18].

In describing the model (Figure 1) more explicitly, known and unknown cellular sources of H_2O_2 are depicted,

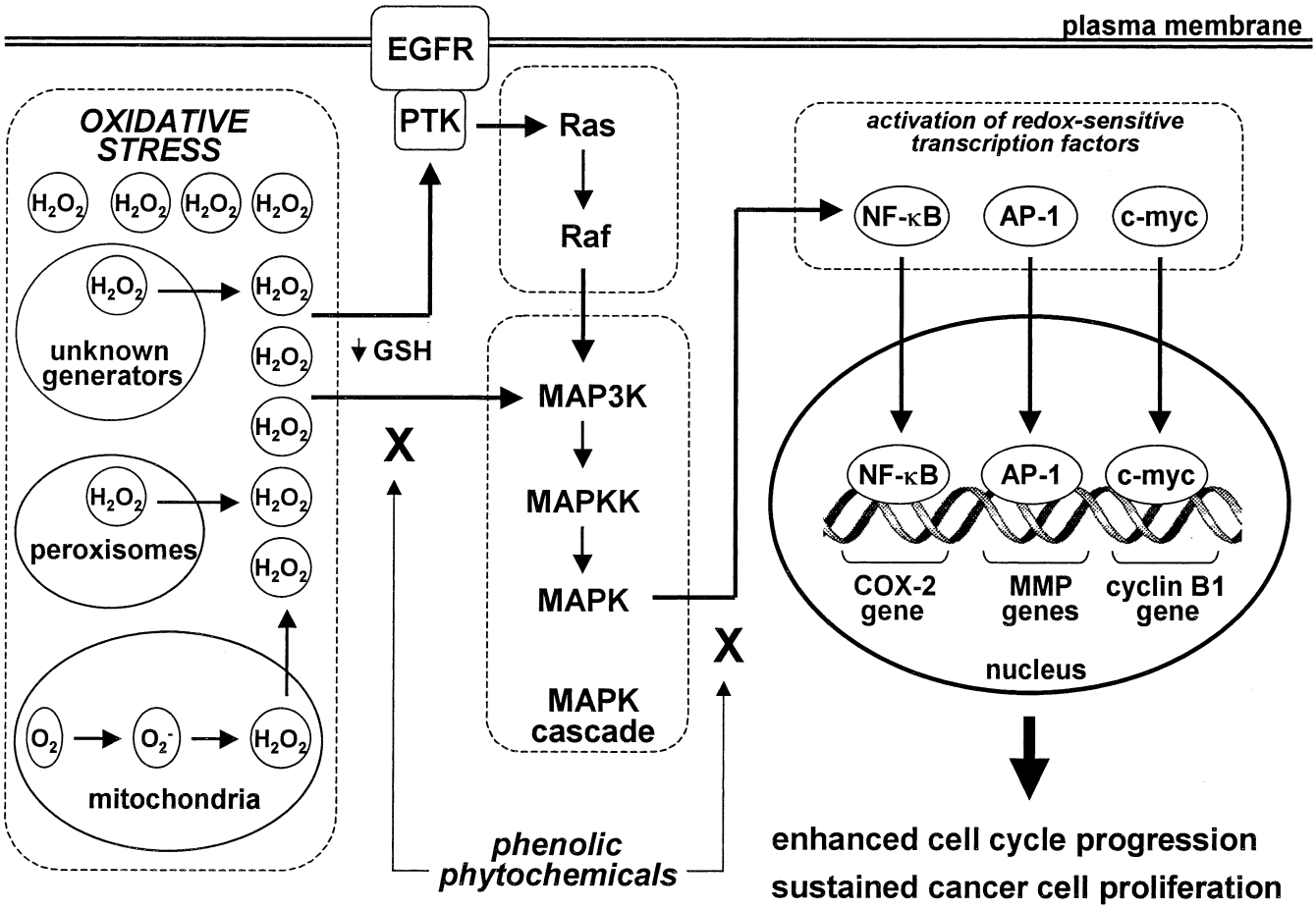

Fig. 1. Putative role of hydrogen peroxide (H_2O_2) in the proliferation of cancer cells and potential impact of phenolic phytochemicals. Oxidative stress in cancer cells due to constitutive high production of tolerable amounts of H_2O_2 overactivate the mitogen-activated protein kinase (MAPK) signaling pathway, resulting in constant activation of redox-sensitive transcription factors and responsive genes that promote cancer cell viability. Phenolic phytochemicals scavenge the H_2O_2 or inhibit protein phosphorylation, thereby disrupting the flow of events leading to cell cycle progression and cancer cell proliferation. See text for further details.

but the largest contributor to the basal or constitutive oxidative stress in cancer cells has not been identified [5]. It is likely that a substantial amount of the H_2O_2 is transformed into $^{\cdot}OH$ in the presence of metal ions. Perhaps by oxidizing key cysteinyl sulfhydryl groups of upstream protein kinases to activate them, or of protein tyrosine phosphatases to inactivate them [19], the constant stream of H_2O_2 or $^{\cdot}OH$ keeps triggering the signaling events. Furthermore, although binding of mitogens such as epidermal growth factor (EGF) to its receptor (EGFR) produces H_2O_2 [20], the large amounts of H_2O_2 constitutively generated by cancer cells increases protein tyrosine kinase (PTK)-mediated phosphorylation or activation of EGFR. Indeed, it is known that H_2O_2 induces phosphorylation of EGFR [21]. This effect on EGFR was inhibited by N-acetylcysteine (NAC), which can bolster intracellular levels of glutathione (GSH). Thus, it seems that the thiol redox status may regulate EGFR phosphorylation. That is, with oxidation of GSH by H_2O_2 in cancer cells, the lowered concentrations of GSH allow activation of the initial signaling events. Consistent with this notion, oxidative stress that was induced by depleting intracellular GSH with N-ethylmaleimide resulted in the accumulation of H_2O_2 and phosphorylation of EGFR [22]. Since catalase and antioxidants prevented these effects, it was concluded that depletion of GSH occurred initially, followed by H_2O_2 accumulation and then EGFR phosphorylation.

Next, according to the model (Figure 1), EGFR activation initiates the Ras signaling cascade that in turn activates the MAPK cascade. Thus, the high amounts of H_2O_2 in cancer cells either directly or through PTK-EGFR-Ras overactivates MAPK, *i.e.*, one or more of its three family members: extracellular signal-regulated kinases (ERK), stress-activated protein kinases (SAPK) that are also referred to as c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinases (p38 MAPK). In support of this scheme, exposing pancreatic acinar cells to H_2O_2 activated all three MAPK [23]. The MAPK activation may have been due to depletion of GSH because the effects of H_2O_2 were prevented by NAC. Interestingly, MAPK activation is increased in tumor tissue, such as papillary thyroid carcinomas [24], compared to normal adjacent tissue. Because the

PTK-Ras-MAPK sequence of events is active in human tumors, this signaling region is a potential target for cancer chemotherapy [25].

Finally, according to the model (Figure 1), the overactivated MAPK overactivates transcription factors including nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which are redox-sensitive [26,27]. NF-κB and AP-1 are required to maintain the phenotype of transformed cells [28,29]. They also are associated with the stimulation of cancer cell proliferation. For example, human androgen-independent DU145 prostate cancer cells have a higher rate of proliferation than androgen-dependent LNCaP prostate cancer cells [30]. The former in contrast to the latter cells expressed activated NF-κB and AP-1 constitutively.

Regarding transcription, NF-κB can activate cancer-relevant genes, such as the COX-2 gene, that is known to have a regulatory DNA sequence in its promoter that binds NF-κB. Consistent with the sequential signaling steps, activated JNK, p38 MAPK, and NF-κB have been detected immunohistochemically in human colon adenomatous polyps [31]. Cellular localization of the activated NF-κB coincided with that of COX-2. Additionally, NF-κB regulated COX-2 gene expression, as well as cellular proliferation, in human gastric cancer AGS cells [32]. However, other redox-sensitive transcription factors such as AP-1 can also mediate COX- 2 gene expression [33].

NF-κB is constitutively activated in cancer cells, such as in Hs294T melanoma cells that can be explained partly by their expression of a NF-κB-inducing kinase/MAPK pathway [34]. Cancer cells with greater metastatic capability have greater NF-κB levels. There is higher constitutive expression of NF-κB in highly invasive MDA-MB-231 human breast cancer cells than minimally invasive MCF-7 human breast cancer cells [35]. Apparently, NF-κB activity is critical for cancer cell viability. Inhibiting NF-κB in mouse adenocarcinoma CSML-100 cells led to apoptosis [36].

In considering AP-1 (c-jun/c-fos) as a transcription factor, it is involved in allowing malignant cancer cells to invade and metastasize by activating MMP genes. Various members of the MMP gene family have AP-1 binding elements in their promoter regions [17], which helps explain why AP-1 via MAPK signaling is involved in activating the MMP-9 collagenase gene [37]. Another redox-sensitive transcription of great importance is c-myc, a proto-oncoprotein that targets multiple genes including those that stimulate cancer cell proliferation [38]. Recently, serial analysis of gene expression (SAGE) revealed that the cell cycle control gene cyclin B1, which is overexpressed during carcinogenesis [39], is a direct target of c-myc [40].

Therefore, according to the overall scheme of events, H_2O_2 has a pivotal role in functioning as a proliferator of cancer cells, whose greater energy metabolism ensures a large continuous supply of H_2O_2 for this purpose. But, can the very molecule that permits cancer cells to thrive also cause their demise?

4. Response of cancer cells to additional oxidative stress

While the constitutive high production of H_2O_2 in some cancer cells appears to promote their proliferation, additional amounts of H_2O_2 above a certain threshold cause cell cycle arrest and/or apoptosis. Depending on the H_2O_2 concentrations, there are different effects on MAPK activation. Which specific MAPK is activated may control the cellular response. When U937 human leukemic cells were exposed to low concentrations of exogenous H_2O_2 (20 μM), there was increased phosphorylation or activation of p38 MAPK that subsequently resulted in cell cycle arrest [41]. These effects by H_2O_2 were prevented by NAC, suggesting that GSH depletion triggered the p38 MAPK phosphorylation. JNK was unaffected, and apoptosis did not happen. The p38 MAPK activation appeared to prevent apoptosis, since inhibition of p38 MAPK with a specific chemical inhibitor permitted the cells to undergo apoptosis. Thus, p38 MAPK activation was needed for survival of the U937 cells exposed to relatively low concentrations of exogenous H_2O_2. In contrast, exposing U937 cells to 1 mM H_2O_2 activated JNK and upstream MAPKK that resulted in apoptosis [42]. However, pretreatment of the cells with 50 μM H_2O_2, which did not activate JNK, prevented activation of JNK by 1 mM H_2O_2. Therefore, pretreatment of the cells with a low dose of H_2O_2 blocked activation of JNK induced by a high dose of H_2O_2. These findings suggested that JNK is involved in initiating apoptosis. Consistent with this suggestion, exposing HT-29 colon cancer cells to H_2O_2 led to activation of ERK, JNK, and p38 MAPK and subsequently caused apoptosis [43]. But, JNK was involved in triggering the apoptosis since a specific chemical inhibitor of JNK prevented the apoptosis. Thus, to what extent the three MAPK are increased by endogenous and/or exogenous H_2O_2 might determine whether cancer cells proliferate, undergo cell cycle arrest, or die by apoptosis.

The persistent oxidative stress in cancer cells apparently sensitizes them to the stress or apoptotic effects of anticancer drugs, which often generate ROS. That is, compared to normal cells, cancer cells are more susceptible to being killed by anticancer drugs because the cancer cells are already near a threshold for tolerating ROS. It is known that NIH 3T3 cells transformed with the Ras oncogene are more sensitive to oxidative stress and genotoxins such as cis-platin [6], which was concluded to be due to potentiation of the JNK/p38 MAPK pathways. The cis-platin increased ROS production where both mitochondria and NADPH oxidases were implicated. Possibly because of similar reasons, cancer cells are more sensitive to killing by phytochemicals as will be discussed below.

In reflecting back on the first four sections of this review, H_2O_2 either stimulates or inhibits the proliferation of cancer cells depending on the concentrations of H_2O_2 present. Accordingly, modulating the concentrations of H_2O_2 in cancer cells could be a means to control their proliferative

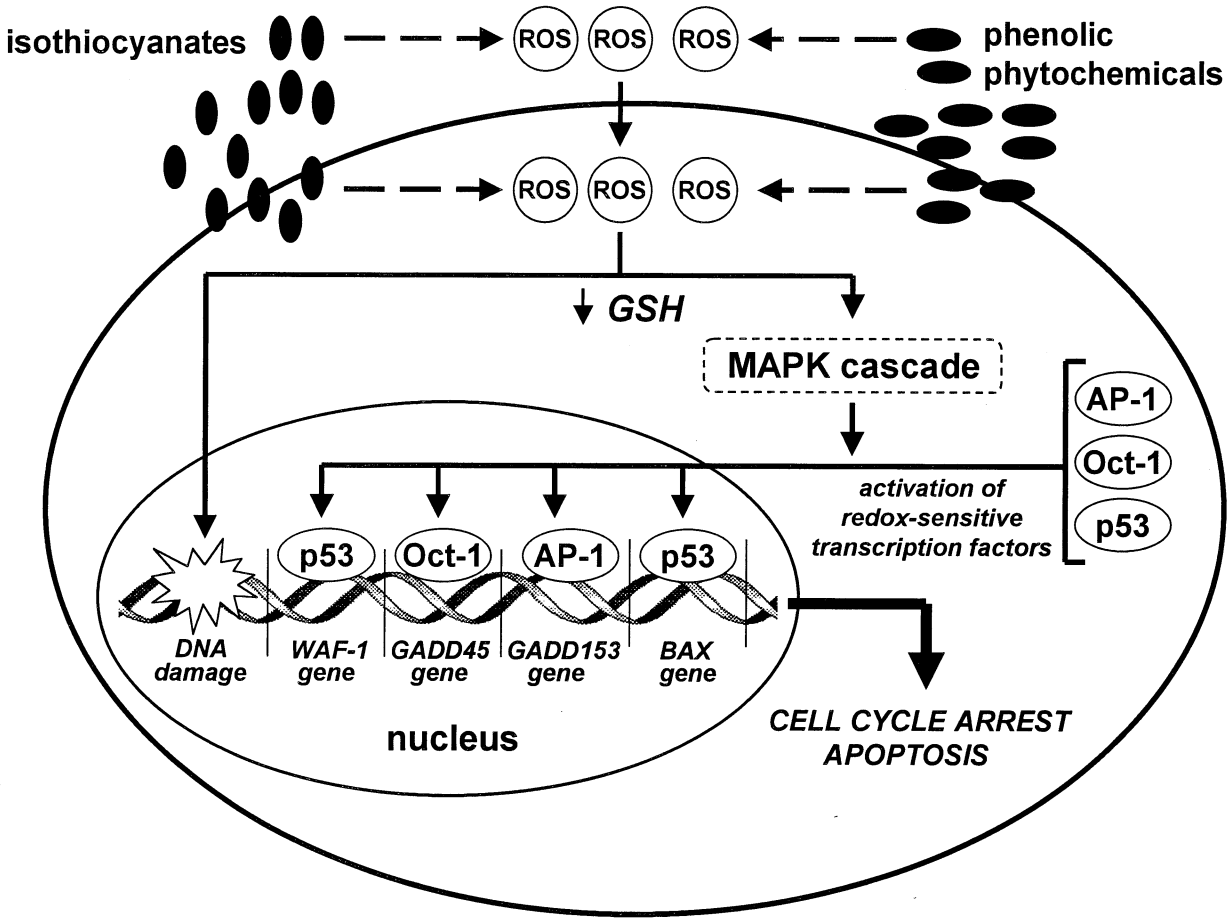

Fig. 2. Concept of how phenolic phytochemicals and isothiocyanates inhibit cancer cell proliferation by inducing the formation of intolerable amounts of reactive oxygen species (ROS). ROS cause irreparable DNA damage as well as activation of stress/survival/death genes that result in cell cycle arrest and/or apoptosis. See text for further details.

activity. How certain phytochemicals modulate the levels of H_2O_2 to control cancer cell proliferation will be considered next as the highlight of this review.

5. Significance of ROS in the inhibition of cancer cell proliferation by phenolic phytochemicals and isothiocyanates

In theory, the apparent reliance of cancer cells on the basal H_2O_2-induced oxidative stress for their vitality provides a logical approach to inhibit their proliferation with antioxidants that scavenge H_2O_2. That is, if the H_2O_2 functions as a secondary messenger in triggering signaling events leading to the activation of cancer cell proliferation genes, then scavenging the H_2O_2 and/or inhibiting protein phosphorylation with phenolic phytochemicals having antioxidant activity should short-circuit the signaling events (as Figure 1 lastly shows) and eventually inhibit cancer cell proliferation.

On the other hand, it turns out that phytochemicals can paradoxically induce oxidative stress to extents that are growth-inhibitory or lethal to cancer cells. From this perspective, Figure 2 conceptualizes how phytochemicals such as phenolic phytochemicals and isothiocyanates inhibit cancer cell proliferation by inducing intolerable oxidative stress in cancer cells or inducing oxidative stress above and beyond that already present under basal conditions. In any event, the intolerable amounts of ROS cause oxidative damage to DNA in conjunction with activation of the MAPK cascade and in turn of growth arrest and DNA damage–inducible genes (such as GADD45 and GADD153), as well as genes that are involved in initiating cell cycle arrest (such as WAF-1), and apoptosis (such as BAX). How phenolic phytochemicals mediate these processes will be considered first, followed by what isothiocyanates do.

5.1. How phenolic phytochemicals inhibit cancer cell proliferation

A recognized property of phenolic phytochemicals is that they have antioxidant activity [1], which is primarily attributable to the phenolic hydroxyl groups being able to furnish

hydrogen atoms in scavenging ROS. The idea has been developed above that H_2O_2, or the reaction product $^{\cdot}OH$, is essential for signal transduction and activation of specific genes that promote cancer cell proliferation. As such, scavenging these ROS with phenolic phytochemicals should inhibit these cellular processes and thus cancer cell proliferation.

Phenolic phytochemicals can inhibit the effects of H_2O_2 on MAPK signaling events and activation of redox-sensitive transcription factors, such as NF-κB and AP-1. In some recent examples of studies, the tea phenolic, epigallocatechin gallate (EGCG), inhibited MAPK activation that was induced in human epidermal keratinocytes by exposing the cells to ultraviolet (UV-B) radiation [44]. The UV-B caused the intracellular formation of H_2O_2 and increased the phosphorylation of ERK, JNK, and p38 MAPK. Because the standard antioxidant, ascorbate, also inhibited the effects of UV-B, it was concluded that EGCG inhibited the UV-B-induced activation of the MAPK by scavenging the H_2O_2. Resveratrol, a phenolic present at high amounts in grapes, prevented NF-κB activation that was induced by tumor necrosis factor (TNF) in U-937 cells, Jurkat cells, HeLa cells, and H4 glioma cells [45]. TNF also stimulated the formation of ROS and activation of MAPKK and downstream JNK, but these effects were inhibited by resveratrol. Additionally, resveratrol prevented NF-κB activation induced by phorbol myristate acetate (PMA), lipopolysaccharide, okadaic acid, ceramide, and most importantly, H_2O_2.

Resveratrol had similar effects on events leading to transcription factor activation, as with the case of AP-1 [46]. Exposing HeLa cells to either PMA or ultraviolet (UV-C) radiation increased transcription of a reporter gene construct containing AP-1 response elements. Pre-treating the cells with resveratrol decreased the transcriptional activation of the construct. Reseveratrol pre-treatment also inhibited activation of ERK, JNK, p38 MAPK caused by PMA and UV-C. In linking these findings, overexpression of dominant-negative mutants of the MAPK in the cells attenuated AP-1 activation induced by PMA and UV-C. The effects of resveratrol were suggested to involve inhibition of both protein tyrosine kinases and protein kinase C, because selective inhibitors of these enzymes inhibited MAPK and AP-1 activation.

Therefore, such studies [44–46] suggest that phenolic phytochemicals can scavenge the constitutively high amounts of H_2O_2 in cancer cells, thereby blocking MAPK signaling, activation of NF-κB and AP-1, and ultimately the expression of responsive genes that stimulate cancer cell proliferation. Because the sustained activation of NF-κB and AP-1 in cancer cells maintains a phenotype favoring their proliferation [28,29], suppressing the activity of these transcription factors with phenolic phytochemicals would be expected to thwart the ability of cancer cells to thrive.

Numerous studies have established that phenolic phytochemicals inhibit the proliferation of cancer cells by inducing cell cycle arrest and/or apoptosis, which might be due to scavenging by the phenolic phytochemicals of H_2O_2 or other ROS needed by cancer cells for their vitality or viability. For example, the phenolic phytochemical, apigenin, induced growth inhibition of human anaplastic thyroid carcinoma cells that was later followed by apoptosis [47]. There was inhibition of EGFR tyrosine autophosphorylation and downstream phosphorylation of MAPK. Thus, in exerting its effects, apigenin may have directly inhibited the protein phosphorylation, or alternatively, scavenged H_2O_2 that activates the protein kinases. Resveratrol induced G_1-phase cell cycle arrest in human epidermoid carcinoma A431 cells [48]. There was decreased protein expression of cyclins and cyclin-dependent kinases (CDK) along with reduced CDK activity that normally mediate cell cycle progression. Consequently, expression of the CDK inhibitor, p21, increased so that apoptosis was the end result. It has been suggested that resveratrol induces apoptosis via JNK activation that leads to phosphorylation and hence activation of p53 [49], which is a transcription factor that activates the Waf-1 gene, which codes for p21, or the BAX gene, whose translated product is known to promote apoptosis. Other phenolic phytochemicals such as quercetin and genistein induced apoptosis in pancreatic carcinoma cells, where mitochondrial depolarization, cytochrome c release, and caspase activation were early apoptotic events [50]. Therefore, since the phenolic phytochemicals have antioxidant activity, it is logical to think that they induced cell cycle arrest and apoptosis in cancer cells by scavenging H_2O_2, thereby depriving them of an essential molecule needed for their existence.

On the other hand, it can be questioned whether phenolic phytochemicals use their antioxidant properties to inhibit the proliferation of cancer cells. Although phenolic phytochemicals unequivocally have antioxidant activity [1], they can paradoxically have prooxidant activity under certain experimental conditions. That is, some are able to generate ROS. EGCG, quercetin, and gallic acid each generated H_2O_2 in time- and concentration-dependent manners when added to cell culture media [51]. Thus, treating cultured cancer cells with these and possibly other phenolic phytochemicals can result in substantial extracellular production of H_2O_2 that can diffuse into the cells and cause stressful or cytotoxic effects. Regarding cellular stress, treating MCF-7 human mammary carcinoma cells with 15 μM quercetin for 24 hr induced transcriptional activation, via the antioxidant response element, of the NAD(P)H:quinone oxidoreductase gene [52], which has an important role in phase II detoxification. However, apoptosis occurred in Ha-ras gene-transformed human bronchial epithelial cells after being treated with 25 μM of EGCG or related tea catechins for 24 hr [53]. Death of the cells was attributed to H_2O_2 because the catechins induced formation of H_2O_2 and the apoptosis was prevented by catalase. Consistent with the view that H_2O_2 was involved, the tea catechins decreased c-jun protein phosphorylation, which would be expected to lower AP-1 activity needed to transcriptionally activate some genes that

promote cancer cell viability. The apoptosis induced by EGCG in human oral squamous carcinoma cells [54] was also concluded to be due to the generation of H_2O_2 in the cell culture medium. Incidentally, the ability of a non-lethal dose of EGCG to increase MAPK-dependent COX-2 gene expression in RAW-264.7 macrophages [55] could be explained by the formation of additional tolerable amounts of H_2O_2 by EGCG.

The induction of intolerable amounts of ROS in cancer cells by phenolic phytochemicals initiates apoptosis through MAPK activation. This is similar to what has been found with other inducers of apoptosis. For example, exposing U-937 promonocytic cells to the toxic heavy metal, cadmium, resulted in the accumulation of H_2O_2 and also phosphorylation of p38 MAPK, followed by apoptosis [56]. Tea catechins including EGCG activated MAPK before initiating caspase-mediated apoptosis [57]. Resveratrol induced apoptosis in thyroid carcinoma cell lines [58]. There were increases in p53 protein, which was mediated by the Ras/MAPK kinase/MAPK pathway.

The induction of apoptosis in cancer cells by some phenolic phytochemicals such as EGCG may be related to oxidative damage to DNA. EGCG [59], as well as curcumin [60], induced single-strand breaks in DNA of Jurkat T-lymphocytes. EGCG increased the formation of H_2O_2 in the cell culture media but curcumin did not [60], suggesting that curcumin was taken up by the cells and then induced the formation of ROS intracellularly. If such oxidative DNA damage is irreparable, apoptosis would be anticipated.

The capacity of phenolic phytochemicals to generate ROS in amounts sufficient to kill cancer cells raises the question whether normal cells are equally susceptible to the prooxidant effects of phenolic phytochemicals. Relevant to this question, compared to normal cells, cancer cells are more susceptible to being killed by anticancer drugs perhaps because the cancer cells are already near a threshold for tolerating ROS. For example, NIH 3T3 cells transformed with the Ras oncogene are more sensitive to both oxidative stress and anti-cancer drugs such as cis-platin [6]. Theaflavin monogallates inhibited the growth of Caco-2 colon cancer cells but not their normal counterparts [61]. They also induced apoptosis in SV40-transformed WI38 human cells but not untransformed WI38 cells. Human oral squamous carcinoma cells and salivary gland tumor cells are more susceptible than normal human gingival fibroblasts to apoptosis induced by the phenolic phytochemicals known as tannins [62].

In light of the above, it is unclear whether phenolic phytochemicals inhibit proliferation of cancer cells using just their antioxidant power alone or their prooxidant power alone. In contrast, it appears that phytochemicals lacking direct antioxidant activity can inhibit cancer cell proliferation by relying just on their prooxidant effects, as the case with isothiocyanates discussed below.

5.2. How isothiocyanates inhibit cancer cell proliferation

In contrast to phenolic phytochemicals, isothiocyanates such as benzyl isothiocyanate (BITC), phenylethyl isothiocynate (PEITC), and phenylmethyl isothiocyanate (PMITC) do not have any significant antioxidant activity. However, isothiocyanates may inhibit the proliferation of cancer cells in an indirect manner similar to the prooxidant action of phenolic phytochemicals. Cultured cells can accumulate high concentrations of some isothiocynates, reaching 800-900 μM after only 30 min of incubation with 5 μM of the isothiocyanates [63]. With such a massive isothiocyanate accumulation, cellular effects would be expected. Apparently, a stress/defense response is triggered in an effort made by the cancer cells to cope and survive. Phase II detoxification enzymes such as NAD(P)H:quinone oxidoreductase are induced by isothiocyanates [64]. Additionally, isothiocyanates are substrates for glutathione-S-transferases, which catalyze covalent bonding of the sulfur atom of glutathione to the electrophilic central carbon atom of the isothiocyanate group to form dithiocarbamate products [65]. It follows that isothiocyanates induce glutathione-S-transferases, which may be under redox regulation [66]. Intracellular glutathione levels also become elevated [64] to allow the conjugation reaction with the isothiocyanates. Thus, cancer cell proliferation, which may be promoted by low concentrations of glutathione as discussed above, would be preferentially arrested in the presence of elevated concentrations of glutathione to permit the stressed cancer cells to detoxify the isothiocyanates that threaten their viability.

As was the case for some phenolic phytochemicals described above, isothiocyanates induce oxidative stress in cells. Incubating HepG2 hepatoma cells with BITC for 3 hr resulted in a dose-dependent increase in the formation of thiobarbituric acid-reactive substances [67], which is a measure of lipid peroxidation caused by ROS. Furthermore, the BITC caused DNA damage that was diminished by antioxidants such as α-tocopherol, ascorbate, and β-carotene. The effect of BITC on ROS generation has also been reported. The level of ROS detected with the fluorescence reagent, 2′,7′-dichlorofluorescin diacetate, was about 50-fold higher in BITC-treated rat liver epithelial RL34 cells compared to untreated cells [66].

The formation of ROS in cells exposed to isothiocynates would be anticipated to have multiple consequent effects. The isothiocyanate known as sulforaphane induced cell cycle arrest in HT-29 human colon cancer cells that was associated with increased expression of cyclins A and B1 [68]. A panel of assays determined that apoptosis subsequently occurred and in the absence of any change in p53 protein levels. In contrast, sulforaphane increased p53 and also the pro-apoptotic protein, Bax, in Jurkat T-lymphocytes [69]. Apparently, p53 is not involved in PEITC-induced apoptosis, based on the report that PEITC induced single-strand breaks in DNA and subsequent apoptosis in HCT-

116 (wild-type p53 gene) and HCT-15 (mutant p53 gene) human colon adenocarcinoma cells (see footnote). However, in normal cells, there is an essential role of p53 in PEITC-induced apoptosis because PEITC induced apoptosis in normal mouse embryo fibroblasts (p53 +/+) but not p53-deficient embryo fibroblasts (p53 -/-) [70]. Continuing with the study on the sulforaphane-induced apoptosis in HT-29 cells [69], there was increased expression of Bax, which was associated with cytochrome c release and cleavage of poly(ADP-ribose)polymerase. These events suggested that caspases were activated to execute the cells. Consistent with this thought, PEITC and structural counterparts each induced apoptosis in HeLa cervical cancer cells that was linked to caspase-3 activation, since a caspase-3 inhibitor (Ac-DEVD-CHO) inhibited the apoptosis [71]. More recent research suggests that generation of ROS and depletion of GSH are important in mediating BITC-induced apoptosis that involved caspase-9 activation and the mitochondrial death pathway [72].

Signaling events leading to apoptosis are initiated in cancer cells treated with isothiocyanates. There is ample support for a role of JNK in mediating isothiocyanate-induced apoptosis and that a reduction in GSH levels triggers the JNK activation [73]. At 5 μM, PEITC and PMITC each increased JNK activity in human leukemic Jurkat T-lymphocytes after 2 hr of incubation. JNK activity remained higher than untreated cells for 12 hr, after which chromosomal DNA laddering was observed that is characteristic of caspase-mediated apoptosis. Furthermore, using human kidney 293 transformed cells, it was further found that transfecting these cells with dominant-negative mutant plasmids of either MEKK or JNK, but not a dominant-negative mutant plasmid of p38 MAPK or a wild-type JNK plasmid, suppressed PEITC-induced apoptosis. The signaling events leading to apoptosis seemed to be triggered by a decrease in intracellular thiols such as GSH, since two thiol-preserving reagents, namely, 2-mercaptoethanol and NAC, inhibited PEITC-induced apoptosis in Jurkat cells. Similarly, PEITC and allyl isothiocyanate induced apoptosis that was associated with JNK activation in human leukemic HL-60 cells [74]. These effects were prevented by fortifying the cells with GSH before exposing them to the isothiocyanates. Therefore, it can be concluded from both of these studies [73,74] that a reduction in the levels of intracellular GSH caused by the isothiocyanates allows the critical signaling events leading to apoptosis.

In considering other molecular effects of isothiocyanates, there is cross-signaling that enables the MAPK pathway to mediate activation of stress/defense genes that are responsive to oxidative stress, as can be induced by isothiocyanates [66,67]. For example, BITC increased reactive oxygen intermediates in association with expression of glutathione-S-transferase mRNA and protein [66]. The growth arrest and DNA damage-inducible genes (GADD) may also be activated, which typically respond to oxidants that trigger signal transduction. Along this line, induction of GADD45 in HCT-116 cells by DNA-damaging UV-radiation involved the MAPK pathway [75]. Inhibition of JNK and ERK by expression of a dominant-negative mutant and by a chemical inhibitor, respectively, inhibited UV-radiation-induced activation of a GADD45 gene promoter/CAT reporter construct. Obviously, transcriptional activation happened because redox-sensitive transcription factors were activated. One transcription factor that is activated as a result of DNA damage is OCT-1 [76], which in addition to NF-YA and p53, can transcriptionally activate GADD45 [77]. Either thiol-reactive agents or ROS generators activated GADD153 gene expression in HeLa cervical cancer cells, which was due to AP-1 activation [78]. Therefore, these findings help explain why PEITC increased GADD34, GADD45, and GADD 153 mRNA levels in colon adenocarcinoma cells [see previous footnote].

6. Conclusions

Some cancer cells constitutively produce high but tolerable amounts of ROS in the form of H_2O_2, which is required for optimum viability. The H_2O_2 depletes GSH and permits overactivation of protein kinases, that in turn overactivate redox-sensitive transcription factors and responsive genes that promote cancer cell survival, growth, and proliferation. Phenolic phytochemicals scavenge the H_2O_2, thereby depriving the cancer cells of an essential second messenger. Consequently, cell cycle arrest or apoptosis occurs. On the other hand, exposing cancer cells to either phenolic phytochemicals or isothiocyanates can induce the generation of intolerable amounts of H_2O_2, which initiates apoptosis. The effects of phenolic phytochemicals and isothiocyanates on cancer cells depend on the dose. It is thought that low concentrations of isothiocyanates and phenolic phytochemicals activate MAPK that mediate the expression of defensive and survival genes such as glutathione-S-transferases and NAD(P)H:oxidoreductase, whereas higher concentrations of these potential cancer chemopreventive substances result in caspase-mediated apoptosis [79,80]. In any event, it is unknown if phenolic phytochemicals and isothiocynates kill cancer cells without depending on either their antioxidant or prooxidant effects.

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