JOURNAL OF CANCER PREVENTION
VOL. 21, NO. 1, MARCH 2016

http://dx.doi.org/10.15430/JCP.2016.21.1.13
pISSN 2288-3649 · eISSN 2288-3657
www.jcpjournal.org

REVIEW

Downregulation of Reactive Oxygen Species in Apoptosis

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Generation of reactive oxygen species (ROS) by diverse anti-cancer drugs or phytochemicals has been closely related with the induction of apoptosis in cancers. Also, the downregulation of ROS by these chemicals has been found to block initiation of carcinogenesis. Therefore, modulation of ROS by phytochemicals emerges as a crucial mechanism to regulate apoptosis in cancer prevention or therapy. This review summarizes the current understanding of the selected chemical compounds and related cellular components that modulate ROS during apoptotic process. Metformin, quercetin, curcumin, vitamin C, and other compounds have been shown to downregulate ROS in the cellular apoptotic process, and some of them even induce apoptosis in cancer cells. The cellular components mediating the downregulation of ROS include nuclear factor erythroid 2-related factor 2 antioxidant signaling pathway, thioredoxin, catalase, glutathione, heme oxygenase-1, and uncoupling proteins. The present review provides information on the relationship between these compounds and the cellular components in modulating ROS in apoptotic cancer cells.
(J Cancer Prev 2016;21:13-20)

Key Words: Reactive oxygen species, Apoptosis, Neoplasms

INTRODUCTION

The increase of reactive oxygen species (ROS) is often observed in the progress of apoptosis,[1,2] and the generation/ upregulation of ROS can be an indication of apoptosis. Indeed, many anticancer drugs and natural compounds (curcumin,[3] garlic,[4] quercetin,[1] cisplatin,[5] etc.) have been known to increase the level of ROS as they induce the apoptosis in cancer cells. While the increase of ROS usually correlates with the apoptotic progress, the downregulation of ROS in apoptotic cancer cells is often observed. While it may seem perplexing at first glance, it is plausible considering the beneficial and essential roles of ROS in physiological conditions.

In this review, we have summarized the current understanding of the selected chemical compounds (Fig. 1) and the related cellular components which modulate ROS during the apoptotic process (Fig. 1). The studies on the apoptotic or anti-apoptotic effects of metformin, quercetin, curcumin, vitamin C, and other compounds in diverse cancer cells are presented (Table 1), followed by the cellular components, including nuclear factor-erythroid2-related factor 2 (Nrf2) antioxidant signaling pathway, thioredoxin (TRX), catalase, glutathione (GSH), heme oxygenase-1 (HO-1), and uncoupling proteins (UCPs). We focus here on the cell-specific downregulation of ROS by selected chemicals as a modulator of apoptosis.

NATURAL/SYNTHETIC ANTIOXIDANTS THAT DOWNREGULATE REACTIVE OXYGEN SPECIES

1. Metformin

Metformin is an oral antidiabetic medication to treat type 2 diabetes. It enhances insulin sensitivity by increasing glucose uptake and utilization in peripheral tissues. These effects are

Received February 17, 2016, **Revised** February 28, 2016, **Accepted** February 29, 2016

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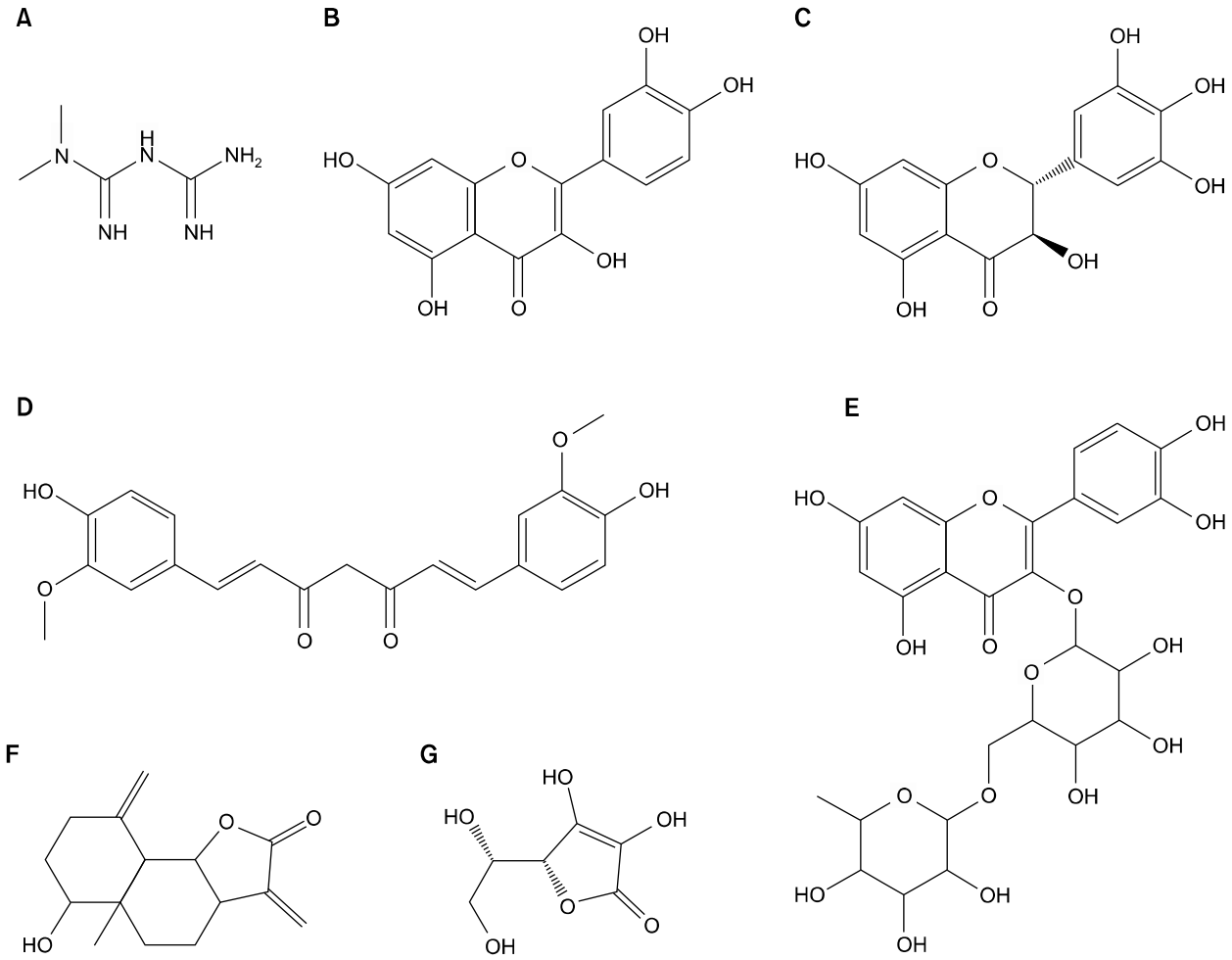

Figure 1. Chemical structures of natural/synthetic compounds. Metformin (A). Quercetin (B). Dihydromyricetin (C). Curcumin (D). Rutin (E). Spirafolide (F). Vitamin C (G).

Table 1. Cell-specific modulation of ROS generation by selected compounds

Compound	Cell type	Effect on ROS	Cellular effect	Reference
Metformin	Renal tubular cells, fatty liver cells	Decrease	Anti-apoptotic	13, 15
Metformin	Pancreatic cancer cells	Decrease	Apoptotic	16
Quercetin	Hepatoma, leukemia	Increase	Apoptotic	24, 25
Quercetin	HUVEC cells	Decrease	Anti-apoptotic	26
Curcumin	Renal, skin, fibroblast, lung adenocarcinoma	Decrease	Anti-apoptotic	3, 30, 31, 32
Curcumin	Neuroblastoma	Increase	Apoptotic	33
Vitamin C	WISH	Decrease	Anti-apoptotic	36
Vitamin C	B16 murine cells	Increase	Apoptotic	38
Spirafolide	Neuroblastoma	Decrease	Anti-apoptotic	39
Retinoic acid	Neuron, cardiomyocyte	Decrease	Anti-apoptotic	41, 42
Retinoic acid	HL60 cells	Increase	Apoptotic	44
Dihydromyricetin	Hepatocarcinoma	Decrease	Apoptotic	45, 46
Dihydromyricetin	Lymphocytes, PC12	Decrease	Anti-apoptotic	47, 48

ROS, reactive oxygen species.

mainly mediated by the activation of AMP-activated protein kinase (AMPK). Quite recently, metformin was suggested to be related with the reduced risk of cancer in diabetic patients.[6,7] While the mechanism by which metformin protects against cancer is veiled yet, metformin has been reported to decrease ROS in several cases. Metformin, at the pharmacological level of $\sim 10^{-5}$ mol/L, lowered the level of ROS in bovine aortic endothelial cells.[8] The production of ROS in endothelial cells, upon glucose-induction, is mainly mediated by several pathways of hyperglycemic responses, including the activation of protein kinase C (PKC).[9] Metformin inhibited the activity of PKC, although it does not have direct inhibitory activity against PKC in vitro. It appears that metformin inhibits the upstream of the activation of PKC, such as membrane translocation of PKC.[10] The AMPK pathway, activated by metformin, increased the expression of the TRX through forkhead transcription factor 3, and the TRX functions as antioxidant to reduce the ROS.[11] The decrease of ROS could reduce the DNA damage, and possibly the risk of cancer.[12] Metformin inhibited the advanced glycation end product-induced apoptosis in renal tubular cells through ROS reduction mediated by the activation of AMPK pathway.[13] The role of the AMPK in the reduction of ROS is not essential, as metformin decreases the ROS even in the AMPK-deficient cells.[14] Moreover, the protective effect of metformin was observed in the fatty liver cell under oxidative stress, probably due to the increased antioxidant enzyme activity, lower ROS production, and reduction of inflammation.[15] While the role of metformin was mostly related with cell protective effects, it was recently reported that metformin decreased the growth of pancreatic cancer cells by reducing ROS production.[16]

2. Quercetin

Quercetin is a widespread flavonoid compound from numerous vegetables and fruits. At least 50 mg of quercetin is estimated to be present in a daily diet.[17] Several clinically relevant functions of quercetin are antihypertensive, anti-inflammatory, hypocholesterolaemic, and antitumor activity.[18] Early studies reported quercetin as a mutagenic compound in the Ames test.[19,20] However, later studies indicated anticancer activity of quercetin.[21,22] Interestingly, quercetin has both pro-oxidative and anti-oxidative properties depending on the redox state of the biological environment.[23] In relation with the apoptosis, quercetin induced the generation of ROS, resulting in apoptosis in hepatoma cells[24] and leukemia cells.[25] Therefore, the pro-apoptotic role of quercetin appears to be related with the upregulation of ROS, not the downregulation. In contrast, the downregulation of ROS by rutin, a quercetin glycoside, was observed in the hydrogen peroxide-induced apoptosis of human umbilical vein endothelial cells.[26]

3. Curcumin

Curcumin is a yellow pigment obtained from the root of the Indian turmeric (*Curcuma longa*). It has been used as a foodstuff, cosmetic, and herbal medicine for a long time. The reported biological activities of curcumin include antioxidant, anti-inflammatory,[27] anticancer,[28] and chemoprevention,[29] etc. Curcumin has been reported to induce apoptosis in numerous cells, including human renal Caki cells,[3] skin squamous cell carcinoma COLO-16,[30] mouse fibroblast L929 cells,[31] and human lung adenocarcinoma A549 cells.[32] In these cases, the induction of ROS mediated the apoptosis. These pro-apoptotic roles of curcumin seem to be involved in the upregulation of ROS, and the downregulation of ROS inhibited the apoptosis of SH-SY5Y cells.[33]

4. Vitamin C

Vitamin C is an essential nutrient in human, and it functions as an electron donor for many enzymatic reactions. It is widely accepted that vitamin C is an antioxidant, and the reduction of ROS by vitamin C treatment has been reported in the TRAIL-induced apoptosis[34] and the oxidized low density lipoprotein (LDL)-induced apoptosis.[35] The reduction of ROS resulted in the protection of the cells from apoptotic damage. On the other hand, vitamin C was not effective in the inhibition of the H_2O_2-induced apoptosis.[36] Interestingly, the H_2O_2-induced apoptosis was preferably exacerbated by vitamin C.[37] Also, vitamin C induced the apoptosis of B16 murine melanoma cells by increasing ROS.[38] Therefore, the role of vitamin C as an antioxidant in the apoptosis is controversial.

5. Other compounds

Spirafolide is a compound purified from the leaves of *Laurus nobilis* L. It has been reported to decrease the ROS level, thereby inhibited dopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells.[39] Fructose, when used as sole carbon source instead of glucose, reduced ROS and stabilized of cellular GSH pool as efficient as N-acetyl-cystein in the oxidative stress-induced apoptosis in liver parenchymal cells.[40]

Retinoic acid, a metabolite of vitamin A metabolism, has been shown to suppress ROS production and inhibit the staurosporine-induced apoptosis.[41] Previous study indicated that treatment with retinoic acid prevented angiotensin II-induced apoptosis in cardiomyocyte by decreasing ROS generation.[42] However, the

upregulation of ROS by retinoic acid was also reported in promyelocytic leukemia,[43] which resulted in apoptosis of granulocyte-differentiated HL60 cells.[44] Consistently, it was accepted that the downregulation of ROS by retinoic acids was related with the prevention of apoptosis.

Dihydromyricetin, a flavonoid compound, was recently shown to induce the apoptosis of human hepatocarcinoma cells by decreasing ROS generation.[45,46] In addition, it is noteworthy that the downregulation of the ROS by dihydromyricetin could block H_2O_2-induced apoptosis of MT-4 lymphocytes[47] and PC12 cells.[48] These results suggest that the downregulation of ROS can differently modulate apoptosis depending on the cell types.

ENDOGENOUS CELLULAR COMPONENTS INVOLVED WITH THE DOWNREGULATION OF REACTIVE OXYGEN SPECIES

1. Nuclear factor erythroid 2-related factor 2 antioxidant signaling pathway

Nrf2 is a basic leucine zipper transcriptional activator.[49] In non-stressed cells, Nrf2 is constantly degraded through ubiquitin-proteasome pathway mainly regulated by Keap1 protein.[50] In the presence of ROS, activated Nrf2 can act as a master regulator of several genes for antioxidant enzymes and detoxifying enzymes by binding activated antioxidant response elements.[51] Those enzymes are NAD(P)H:quinone oxidoreductase (NQO1),[52] glutathione S-transferase,[53] and HO-1.[54] The protective role of Nrf2 signaling pathway in the apoptotic process was evident. Nrf2 mediated the expression of HO-1 and NQO1, thereby protected cells from the Cr(VI) induced-apoptosis.[55] Upregulation of HO-1 by Nrf2 rescued PC12 cells from H_2O_2-induced apoptosis.[56] Moreover, the presence of Nrf2 increased the level of TRX, thereby protected human dopaminergic neuroblastoma SH-SY5Y cells from the paraquat-induced cell death.[57] In addition to antioxidant proteins, Nrf2 also regulated the expression of anti-apoptotic protein Bcl-2.[58] While Nrf2 signaling pathway showed the anti-apoptotic effect in most cases, constitutively active Nrf2 enhanced the apoptosis of damaged liver cells.[59] It might be possible to modulate the activity of Nrf2 to either protect or damage the cells.[60]

2. Thioredoxin

TRX is an oxidoreductase enzyme containing dithiol-disulfide active site.[61] There are TRX isoforms in most organisms, and there exist separate TRX system for cytoplasm and mitochondria. TRX functions as a protein disulfide reductase and an electron donor for other enzymes such as ribonucleotide reductase and peroxidase.[62] Conditional knockout of a mitochondrial enzyme TRX-2 resulted in the induction of apoptosis in chicken B-cell lines, DT40,[63] and overexpression of TRX-2 inhibited the TNF-α-induced apoptosis of HeLa cells,[64] indicating the anti-apoptotic role of TRX. Moreover, TRX inhibited apoptosis signal-regulating kinase 1 (ASK1) by promoting the ubiquitination of ASK1, demonstrating the role of TRX beyond ROS removal.[65] In most cases, TRX has been shown to possess a protective and anti-apoptotic function. However, the pro-apoptotic role of TRX was also reported in the anthracycline-induced apoptosis of MCF-7 breast cancer cells. The expression of the redox-inactive mutant TRX resulted in decreased superoxide generation and apoptosis.[66]

3. Catalase

Catalase is a peroxisomal enzyme that converts hydrogen peroxide, a ROS, into water and oxygen. Inhibition of catalase can result in the increase in ROS and oxidative damage. Indeed, TGF-β1-induced suppression of the expression/activity of catalase caused the apoptosis of hamster pancreatic beta-cell line.[67] On the other hand, the overexpression of catalase could attenuate the apoptosis induced by oxidized LDL stimulation[68] and UV-B radiation.[69] Catalase showed a protective and anti-apoptotic role in most cases by eliminating ROS. Nevertheless, it was also reported that the overexpression of human catalase inhibited proliferation and promoted the apoptosis of vascular smooth muscle cells.[70]

4. Glutathione

GSH is a tripeptide compound containing cysteine present in animal, plant, and fungi. It serves as an antioxidant with the free thiol group of cysteine residue. The oxidized form of GSH (GSSG) contains two GSH with disulfide linkage, and the ratio of GSH vs. GSSG can be a good measure of redox state of the cell.[71] The protective and anti-apoptotic role of GSH was shown in MDBK bovine renal epithelial cells: the selenium-dependent GSH peroxidase (GPx) protected the cells against the H_2O_2-induced apoptosis,[72] whereas the suppression of GPx enhanced the H_2O_2-induced apoptosis.[67] Therefore, GSH depletion is closely correlated with the apoptotic induction in most cases, and the protective and anti-apoptotic role of GSH might be due to its antioxidant function.

5. Heme oxygenase-1

HO-1 is a stress-responsive enzyme catalyzing the degradation of heme into carbon monoxide (CO), biliverdin, and iron (Fe^{2+}).[73] The HO-1-inducing stress stimuli include X-ray-induced oxidative stress,[74] hypoxia,[75] and ultraviolet.[76] All three products of HO-1 reaction serve as antioxidants and have other protective roles against apoptosis. Pharmacological upregulation of HO-1 prevented the glutamate-induced apoptosis of cerebral vascular endothelial cells.[77] Upregulation of HO-1 protected human keratinocyte (HaCaT) cells against UV-A–induced oxidative stress.[78] However, it does not always imply that the induction of HO-1 plays a protective role in cells against apoptosis, considering that induction of HO-1 increased in the Nickel (II)-induced apoptosis of human Jurkat cells.[79] This might be resulted from the response of cells to the increased level of ROS. It appears that the expression of HO-1 has to do with the reduction of ROS as seen in the dihydromyricetin-induced apoptosis of human hepatoma HepG2 cells.[45]

6. Uncoupling proteins

UCPs are mitochondrial inner membrane proteins, and they dissipate proton gradient. The physiological roles contain heat generation as in hibernation, cold exposure, and normal body temperature. In addition, UCP2 has been shown to modulate the mitochondrial generation of H_2O_2.[80] Splenocytes, resistant to oxidative stress-induced apoptosis, have been reported to show high level of UCP2 expression.[81] Overexpression of UCP2 inhibited ROS generation and blocked the apoptosis in human aortic endothelial cells induced by lysophophatidylcholine.[82] The inhibition of UCP2, on the contrary, exacerbated the apoptosis in kidney cells.[83] Several other reports also supported the anti-apoptotic function of UCP2 through the downregulation of ROS.

CONCLUSION

We have summarized the controversial role of selected natural/synthetic compounds in modulating cell apoptosis by different regulation of ROS generation. Because the cellular components mediating the downregulation of ROS have not been tied up with corresponding compounds yet, further study in this field is required to establish clearer relationship between these compounds and the cellular components in cancer cells. These natural/synthetic compounds can be useful in modulating the apoptotic process, and in providing new strategies in cancer prevention and therapy.

ACKNOWLEDGMENTS

This study was supported by College of Pharmacy-specialized Research Fund (from Institute for New Drug Development) of Keimyung University and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A2054979).

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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