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Signaling Mechanism(S) of Reactive Oxygen Species in Epithelial-Mesenchymal Transition Reminiscent of Cancer Stem Cells in Tumor Progression

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Abstract

Reactive oxygen species (ROS) are known to serve as a second messenger in the intracellular signal transduction pathway for a variety of cellular processes, including inflammation, cell cycle progression, apoptosis, aging and cancer. Recently, ROS have been found to be associated with tumor metastasis involving the processes of tumor cell migration, invasion and angiogenesis. Emerging evidence also suggests that Epithelial-Mesenchymal Transition (EMT), a process that is reminiscent of cancer stem cells, is an important step towards tumor invasion and metastasis, and intimately involved in *de novo* and acquired drug resistance. In the light of recent advances, we are summarizing the role of ROS in EMT by cataloging how its deregulation is involved in EMT and tumor aggressiveness. Further attempts have been made to summarize the role of several chemopreventive agents that could be useful for targeted inactivation of ROS, suggesting that many natural agents could be useful for the reversal of EMT, which would become a novel approach for the prevention of tumor progression and/or the treatment of human malignancies especially by killing EMT-type cells that share similar characteristics with cancer stem cells.

Keywords

Reactive oxygen species; EMT; cancer

THE CONCEPT OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

In recent years, a large body of literature has emerged documenting the biological significance of EMT in tumor progression and drug resistance. It is now widely known that epithelial cells could get converted into mesenchymal cells by a fundamental process defined as EMT. The processes of EMT were originally identified as a crucial differentiation and morphogenetic physiological process during embryogenesis [1], and it is now recognized as pathological mechanism in the progression of various diseases including inflammation, fibrosis and cancer. During EMT, epithelial cells undergo remarkable morphologic changes characterized by a transition from epithelial cobblestone phenotype to an elongated fibroblastic phenotype (mesenchymal phenotype), which leads to enhanced cell motility and invasion. During the acquisition of EMT characteristics, cells lose epithelial characteristics and gain mesenchymal characteristics. Specifically, cells lose epithelial cell-cell junction, actin cytoskeleton reorganization and the expression of proteins that promote

cell-cell contact such as E-cadherin and β -catenin, and gain the expression of mesenchymal markers, such as fibronectin, vimentin, α -smooth muscle actin (SMA), fibrillar collagen (type I and III), fibroblast-specific protein-1, N-cadherin as well as acquired increased activity of matrix metalloproteinases (MMPs) like MMP-2, MMP-3 and MMP-9. Interestingly, EMT is dependent on E-cadherin, and the cells undergoing EMT showed down-regulation of E-cadherin under most circumstances. This cell-cell adhesion molecule is a calcium-dependent transmembrane glycoprotein present in most epithelial cells in adult tissues. E-cadherin transcriptional repression may result from the activation of repressors, such as the zinc finger Snail homologs (Snail1, Snail2/Slug, and Snail3) and several other basic helix-loop-helix transcription factors, such as Twist, ZEB1, ZEB2/SIP1, and TCF3/E47/E12.

Although, EMT was originally identified as a crucial differentiation and morphogenetic process during embryogenesis, it has now been found to be important in cancer progression and metastasis. Recent findings suggest that metastasis may be critically dependent on the ability of cancer cells to acquire EMT characteristics [1,2]. Interestingly, recent findings clearly suggest that “cancer stem cells” may represent reservoir cell types present in a growing tumor mass where the processes of EMT may play a critical role [3]. Recent studies have also shown that EMT is important on conferring drug resistance characteristics to cancer cells against conventional therapeutics including taxol, vincristine and oxaliplatin targeted therapy [4], which is consistent with drug resistance characteristics of “cancer stem cells”. Therefore, discovery of precise mechanism(s) that governs the processes of EMT, and the maintenance of cancer stem cell reservoir (that are typically drug-resistant), would likely serve as newer targets by which novel strategies could be exploited for the inhibition of tumor progression and/or the killing of cancer stem cells in order to achieve complete eradication of tumors. Indeed, very recently, Gupta *et al.* identified a selective agent that kills cancer stem cells (EMT-type cells) very efficiently [5]. They found that salinomycin destroyed cancer stem cells selectively. When compared to paclitaxel, which is a commonly used drug for the treatment of many malignancies including breast cancer, salinomycin reduced the proportion of cancer stem cells by more than 100-folds and inhibited mammary tumor growth in mice, which was associated with increased epithelial differentiation of tumor cells [5]. These provocative results clearly suggested that the EMT-type cells share biological characteristics that are similar to cancer stem cells, hence it is important to understand the role of ROS in cancer stem cells, such as EMT-type cells in experimental system.

Multiple oncogenic pathways mediated by peptide growth factors, Ras, integrin, Wnt and Notch signaling, are known to induce EMT. It is well accepted that EMT is a dynamic process and is triggered by the interplay of extracellular signals and many secreted soluble factors, such as transforming growth factor- β (TGF- β), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factors (PDGF) as well as different isoforms of Wnt proteins or MMPs or bone morphogenetic proteins, Hedgehog, Notch, and nuclear factor- κ B (NF- κ B) signaling pathways. Recent experimental evidence has shown that the presence of hypoxia and the generated reactive oxygen species (ROS) can also induce EMT. Emerging evidence has also implicated the critical role of several microRNAs (miRNAs) in the processes of EMT. Recently, ROS signaling pathway has been reported to be intimately involved with EMT in both physiological conditions and pathological processes, however the functions of ROS in the processes of EMT remain unclear. Therefore, in this succinct review article, we focused our discussion on describing the role of ROS in the processes of EMT, and further suggesting that the readers could gain additional knowledge by reading several excellent reviews in the field of EMT in general [6-12], which will spur active research in this ever emerging field.

BACKGROUND OF REACTIVE OXYGEN SPECIES (ROS)

ROS, continuously generated from mitochondrial respiratory chain, include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen. ROS are produced continuously *in vivo* under aerobic conditions. In eukaryotic cells, the mitochondrial respiratory chain, microsomal cytochrome P450 enzymes, flavoprotein oxidases, and peroxisomal fatty acid metabolism are the most significant intracellular sources of ROS. The NADPH oxidases are a group of plasma-membrane-associated enzymes which catalyze the production of O_2^- from oxygen by using NADPH as the electron donor. Mammalian cells possess an efficient antioxidant defense system, mainly composed of the enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase and peroxidases, and also some small molecules of antioxidants, like glutathione (GSH), which can scavenge the excessive ROS produced through cellular metabolism, and make ROS level relatively stable under physiological conditions in order to maintain cellular homeostasis. Researchers have also suggested that ROS serve as a second messenger in the intracellular signal transduction pathway for a variety of cellular processes, including cell cycle progression, apoptosis, and aging. ROS have been reported to be involved in over 150 human disorders [13]. It is believed that when the intracellular homeostatic mechanism fails then the overproduced ROS could cause cellular oxidative stress, where DNA, lipids, proteins and other cellular components are oxidatively damaged. DNA damage induced by ROS is sufficient to convert normal cells to malignant cells; therefore, aberrant ROS signaling has profound effect in inducing human malignancies [14], suggesting that novel anti-oxidants could be useful for the inhibition of tumor progression.

It has been reported that ROS are emerging as critical signaling stimuli that mediate several cellular functions, including tissue homeostasis and adaptation. ROS can activate various signaling pathways, including Akt/protein kinase B, p42/p44 mitogen-activated protein kinase (MAPK), p38 MAPK, Rho-GTPase, Smads cascades, Ras-Raf-MEK-ERK (extracellular signal regulated kinase), signal transducers and activators of transcription (STAT), and protein kinase C (PKC) [13,15-17]. ROS also affect many other targets, such as nuclear factor- κ B (NF- κ B) and p21 kinase. A number of genes have been shown to be involved in ROS-mediated signaling pathways, including matrix metalloproteinases (MMPs), integrins, EGF, EGFR, VEGF (vascular endothelial growth factor), TGF- β , HIF-1, HGF, NADPH oxidases (nicotinamide adenine dinucleotide phosphate oxidases, Nox), p53, Bcl-2, caspase, etc [13,15-20]. The reason why ROS activate many signaling pathways is that cells under oxidative stress are likely to have acquired adaptive mechanisms to counteract the potential toxic effects of elevated ROS to promote many cell survival pathways and factors. Several factors are redox-sensitive transcription factors, such as NF- κ B, C-Jun, and HIF-1. These factors can promote the expression of cell survival molecules, such as the Bcl-2 family proteins and Akt pathway [16]. ROS are necessary and sufficient to stabilize and activate HIF-1. It is well documented that HIF-1 can regulate many cell survival pathways, such as NF- κ B, VEGF. ROS can also activate upstream signaling of HIF-1, such as PI3K/AKT, ERK and p38 MAPK pathways. Therefore, it has been believed that HIF-1, which is activated by ROS, may act as a chief orchestrator of the events that lead to metabolic adaptation, sustained cell proliferation, inhibited cell apoptosis, promoted tumor progression and aggressiveness [19]. Many excellent reviews have documented the cross-talk between ROS and signaling pathways in cancer [13,15-23]; however, the role of ROS in the processes of EMT or in cancer stem cells remains unclear. Therefore, in this article, we will discuss the effect of ROS in the acquisition of EMT and its biological significance in tumor progression and drug resistance.

THE ROLE OF ROS IN EMT

It is believed that the processes of EMT, an excellent experimental system for understanding the biological characteristics of cancer stem cells, is stimulated and regulated by many factors, such as growth factors, signal transduction pathways and transcription factors. Recently, the involvement of ROS signaling in the processes of EMT has been highlighted. In this report, we have attempted to catalogue how ROS are generated and how ROS could target downstream molecules and thus triggering the processes of EMT.

ROS and TGF β

TGF- β signaling is involved in a vast majority of cellular processes including EMT [24]. The functioning of the TGF- β pathway depends on its constitutive and extensive communication with other signaling pathways, resulting in synergistic or antagonistic effects and thus resulting in the context-dependent desirable biological outcomes. Recent studies have documented a cross-talk between TGF- β and ROS signaling. For example, Rhyu *et al.* reported that TGF- β 1 increased dichlorofluorescein-sensitive cellular ROS, phosphorylated Smad 2, p38 MAPK, ERK1/2, α -SMA expression, and fibronectin secretion and decreased E-cadherin expression. Antioxidants effectively inhibited TGF- β 1-induced cellular ROS, p38 MAPK, ERK and EMT [25]. Inhibitors of NADPH oxidase (diphenyleneiodonium and apocynin) also significantly inhibited TGF- β 1-induced ROS [25]. These results suggest that ROS play a key role in TGF- β 1-induced EMT primarily through activation of MAPK and subsequently through ERK-directed activation of Smad pathway. Very recently, Zhang *et al.* reported that ferritin heavy chain (FHC), a cellular iron storage protein, was a novel modulator of TGF- β 1-induced EMT [26]. Specifically, there was a dramatic decrease in the FHC levels after TGF- β 1 treatment, which caused iron release from FHC and increased the intracellular labile iron pool (LIP). Increased LIP by TGF- β 1 treatment promoted the production of ROS, which in turn activated p38 MAPK [26]. Moreover, the elimination of ROS inhibited EMT, whereas H₂O₂ treatment rescued TGF- β 1-induced EMT in cells in which the LIP increase was abrogated. Furthermore, over-expression of exogenous FHC attenuated the increases in LIP and ROS production, leading to the suppression of EMT. These results suggest that TGF- β 1-induced EMT is dependent on ROS production catalyzed by elevated LIP [26]; hence it is believed that TGF- β is an important factor in inducing the processes of EMT, suggesting that novel inhibitors of TGF- β could be useful for the killing of EMT-like cells or cancer stem cells.

ROS and TNF- α

Many investigators have reported that inflammatory cytokines such as TNF- α , generated by activated monocytes–macrophages, are also known to produce increased amounts of ROS. It has been well accepted that NF- κ B is activated by a variety of stimuli including cytokine TNF- α , radiation, and oxidative stress, suggesting that ROS are potent activator of the “master” transcription factor NF- κ B. It has been reported that TNF- α can promote EMT of MCF-7 breast cancer cells [27] and during this process the expression of Snail and vimentin was increased, whereas E-cadherin was decreased, which is the “hallmark” of EMT. Moreover, the activation of NF- κ B, which causes an increase in the expression of Snail, and was essential for TNF- α -induced EMT [27]. However, the ROS caused by TNF- α seems to play a minor role during the TNF- α -induced EMT although H₂O₂ alone can promote EMT in a way that is known to be different from TNF- α -induced EMT, in which NF- κ B only plays a minor role, suggesting that different mechanisms might be responsible for TNF- α and ROS-induced EMT [27]. These can be explained by the facts that ROS produced by TNF- α are different in quantity, time and compartment from those produced by treatment with exogenous H₂O₂ or under hypoxia or other conditions. In addition, ROS may serve as

activators, bystanders, or inhibitors of EMT in different situations [27]. Recently, it has been demonstrated that ROS-mediated activation of Src kinase resulted in stress fiber formation and cell-cell dissociation through the activation of Rho kinase pathway [28]; however, cell-cell dissociation induced by oxidative stress was transient, due to activation of NF- κ B, and the expression of manganese SOD. It is believed that NF- κ B is a part of a negative feedback loop for controlling intracellular ROS levels because H₂O₂ treatment alone does not induce EMT, whereas TNF- α treatment causes the induction of EMT. These findings suggest that oxidative stress is a crucial factor to induce cell-cell dissociation, an initial step towards the acquisition of EMT characteristics, but does not provide sufficient signals to establish and maintain EMT [28], suggesting that further research in this area is urgently needed.

ROS and Hypoxia Inducible Factor (HIF-1- α)

HIF-1, consists of α and β subunits, is a major transcription factor involved in cell response to hypoxia. Hypoxia is a common feature of many cancers that contributes to local and systemic tumor progression and compromises the tumor killing effects of radiotherapy and chemotherapy, thus it is believed that hypoxia and the activation of HIF-1 plays important roles in cancer stem cell niche, which requires active research in this area. Although low oxygen tension may contribute to the killing of some tumor cells, hypoxia highly provides a strong selective pressure that is able to regulate tumor growth and also initiate the processes of EMT, resulting in tumor aggressiveness and drug resistance. Under hypoxic conditions, cells respond by stabilizing HIF-1 α that, in turn, dimerizes with HIF-1 β , translocates into the nuclei and activates its target genes to sustain several changes that are necessary to efficiently counteract the decrease in oxygen tension. Recently, Cannito *et al.* reported that moderate hypoxic conditions can trigger EMT in different human cancer cells, resulting in increased invasiveness [29]. Hypoxia-dependent changes occur through a biphasic mechanism. For example, at an early stage, ROS-dependent inhibition of glycogen synthase kinase-3 (GSK-3 β), followed by early Snail translocation and down-regulation of E-cadherin, can switch to EMT phenotype, whereas at a later stage, long-lasting activation of Wnt/ β -catenin signaling and VEGF are involved [29]. These findings suggest that early redox mechanisms can turn-on the switch for hypoxia-dependent acquisition of EMT characteristics whereas increased invasiveness is sustained by HIF-1 α -dependent release of VEGF [29], which in-itself is considered a vicious cycle of tumor progression and metastasis.

ROS and TPA (Tumor 12-O-Tetradecanoylphorbol-13-Acetate)

It has been reported that TPA can activate the PKC β -MEK-ERK signaling cascade for induction of p16 expression, leading to growth suppression of human hepatoma cells [30]. In addition, TPA could also trigger EMT in human hepatoma cells. Wu *et al.* reported that ROS play a central role in sustained PKC-ERK signaling during TPA-induced EMT-like cell scattering and migration of hepatoma cells [31], suggesting an intimate relationship between ROS, TPA-mediated signaling and EMT. Specifically, generation of the ROS induced by TPA was found to be PKC-dependent, and that the scavengers of ROS, especially by catalase, SOD, and mannitol significantly suppressed the TPA-triggered cell migration. TPA also shown to induce the expression of integrins and reduce the expression of E-cadherin in a PKC- and ROS-dependent manner [31]. These findings suggest that ROS play a central role in mediating TPA-induced sustained activation of PKC, which down-regulates the expression of E-cadherin, resulting in the acquisition of EMT phenotype, which could be similar in preserving the cancer stem cell niche and the maintenance of the biological characteristics of cancer stem cells reservoirs.

ROS and MMP-3

Matrix metalloproteinases (MMPs) are critically involved in the processes of tumor cell invasion and metastasis. Tumor metastasis occurs by a series of steps including cell invasion, degradation of basement membranes and the stromal extracellular matrix, ultimately leading to tumor cell invasion and metastasis. Overall, the MMPs are a family of related enzymes that degrade extracellular matrix, which are considered to be the important factors in facilitating tumor invasion. It has been reported that increased expression of MMPs is predictive of tumor aggressiveness, metastasis and poor patient survival in many human cancers [32], suggesting that MMPs are critically involved with many aspects of tumor aggressiveness.

Recently, MMPs have been considered to be an important factor in triggering EMT. Expression of MMP-28 was found to be induced during TGF- β mediated induction of EMT in A549 lung adenocarcinoma cells [33]. It has been shown that MMP-3 can also induce EMT and malignant transformation in cultured cells, and in genomically unstable mammary carcinomas in transgenic mice [34]. MMP-3-induced EMT was associated with the loss of intact E-cadherin, increased motility and invasiveness, down-regulation of epithelial markers, and up-regulation of mesenchymal markers. In this process, the authors have shown the involvement of the GTPase Rac [34], suggesting that MMP-3-induced EMT is mediated by the activation of Rac. The Rac signaling is known to be essential for cytoskeletal rearrangement and considered an important molecule in mediating integrin signaling. Several reports have demonstrated that ROS can be generated by integrin-Rac pathway, resulting in tumor cell migration and invasion [35,36]. MMP-3-induced EMT appears to be mediated via induction of ROS and increased expression of the Rac1b [34]. Interestingly, ROS-quenching agent N-acetyl cysteine (NAC) effectively inhibited the MMP-3-induced EMT [34]. These results clearly suggest that the treatment with MMP-3 stimulates the expression of Rac1b, which increases intra-cellular ROS, leading to the induction of EMT, suggesting that MMP-3 inhibitor or the inhibitors of ROS could be useful in the reversal of EMT or the killing of EMT-type cells or cancer stem cells leading to decreased tumor aggressiveness.

ROS and Other Factors

Many factors are involved in the production of ROS, which has been reported to be directly associated with the processes of EMT. For example, HGF is known to induce EMT and migration in a variety of cancer cell lines [37,38], which may involve the complex mechanism of ROS, leading to increased metastatic potential of cancer cells [39]. Recent studies have also shown that EGF could promote pancreatic cancer cell invasion through Rac1/ROS-dependent secretion and activation of MMP-2 [40]. The inhibition of the EGF pathway enhances TGF- β -induced apoptosis in rat hepatoma cells through inducing oxidative stress coincident with a change in the expression pattern of the NADPH oxidases (NOX) isoforms [41]. ROS are known to regulate EGF-induced VEGF and HIF-1 expression through activation of AKT and P70S6K1 in human ovarian cancer cells [42]. Moreover, it has been reported that EGF can trigger EMT in human cancer cell lines [6], suggesting that further in-depth investigation is needed for testing the hypothesis whether ROS play any critical role during EGF-induced EMT.

Emerging evidence suggest that ROS can regulate several microRNA in human cancer [43,44]. Several recent studies have suggested that the miRNAs play important role in the regulation of critical genes that are important during the acquisition of EMT [7]. For example, miR-200 family and miR-205 have been shown to function as key regulators of EMT because the expression of these miRNAs may function as enforcers for maintaining epithelial phenotype. The miR-200 family participates in a signaling network with the E-

cadherin transcriptional repressors ZEB1/deltaEF1 and ZEB2/SIP1. We have recently found that miR-200 regulated the PDGF-D-mediated EMT in prostate cancer cells [45]; however, whether miRNAs are directly associated with ROS-mediated EMT awaits further in-depth investigations. Nevertheless, it is tantalizing to speculate that the regulation of critical genes by miRNAs are important and would become a novel approach by which critical genes could be dysregulated by employing miRNA-targeted agents for selective killing of EMT-type cells or cancer stem cells. Taken together, ROS play an important role in EMT progression as illustrated in Fig. (1).

PREVENTION OF TUMOR PROGRESSION BY ANTIOXIDANT

Since ROS play a central role in tumor progression, which in part could be directly mediated by the acquisition of EMT pheno-type or preserving the cancer stem cell population, chemical or enzymological antioxidants could be useful to reduce oxidative stress and the reversal of EMT and tumor aggressiveness. More importantly, the dietary chemopreventive agents and nutrients that are known for their non-toxic and pleiotropic activity may fulfill this promise by preventing ROS-mediated tumor progression involving the processes of EMT. In the following paragraphs, we have presented the state of our knowledge on how natural or nutritional agents could be useful in the reversal or killing of EMT-type cells or cancer stem cells although this field is in its infancy and requires in-depth research.

The Role of Zinc

Zinc has multiple biochemical roles as an antioxidant. For example, NADPH oxidases are a group of plasma-membrane-associated enzymes which catalyzes the production of O_2^- from oxygen by using NADPH as the electron donor is indeed inhibited by zinc. The dismutation of O_2^- to H_2O_2 is catalyzed by SOD, which contains both copper and zinc, suggesting the importance of these metal ions. Zinc can induce the production of metallothionein, which is very rich in cysteine and is a scavenger of $\cdot OH$. Zinc can compete with both iron and copper for binding to the cell membrane, thus decreasing the production of $\cdot OH$ [46]. We have recently reported that zinc could function as antioxidant in human. For example, zinc supplementation led to down-regulation of the inflammatory cytokines through up-regulation of the negative feedback loop A20 and inhibition of NF- κ B activation [46]. Zinc supplementation is also known to decrease oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients [47]. These results suggest that zinc supplementation may be beneficial for the reduction of ROS; however no studies have shown the role of zinc, if any, in the processes of EMT, which could be an interesting subject area of future research.

The Role of Known Chemopreventive Agents in ROS and EMT

We will restrict our discussion on selective agents in this section. Among many natural chemopreventive agents, soy isoflavone, genistein, a natural isoflavonoid found in soybean products, has been proposed to be associated with lower incidences of pancreatic cancers and is believed to function as a chemopreventive agent [48]. Genistein has been known to compete with estradiol for estrogen receptor binding as weak estrogen, regulate signal transduction as inhibitor of protein tyrosine kinases, and inhibit cellular oxidative stress and angiogenesis [49]. It has been demonstrated that genistein sensitizes leukaemia cells to arsenic trioxide-provoked apoptosis via ROS-mediated activation of p38-MAPK and AMPK [50]. It has been reported that low-dose genistein induced the reversal of EMT and inhibited invasion, suggesting that the treatment with low-dose genistein may be a potential strategy for the suppression of invasive growth through the reversal of EMT in cancer cells [51]. Recently, we found that the treatment of gemcitabine-resistant (GR) pancreatic cancer cells showed EMT phenotype with isoflavone resulted in the down-regulation of ZEB1, slug, and

vimentin, which was consistent with morphologic reversal of EMT phenotype leading to epithelial morphology [52]. These limited studies clearly suggest that further in-depth molecular mechanistic studies are required in order to determine whether ROS are involved in GR-induced EMT or not and how soy isoflavones could be involved in the reversal of EMT phenotype and thus will reduce tumor aggressiveness.

Another important agent is Curcumin (diferuloylmethane), which is a phenolic compound found in the plant *Curcuma longa* (Linn), and is widely used flavoring agent in food. Curcumin has recently received considerable attention due to its pronounced anti-inflammatory, anti-oxidative, immunomodulating and anti-carcinogenic activities [53]. It has been found that curcumin inhibited the nuclear levels of arylhydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT). It was also found that ROS mediated the curcumin-induced degradations of AhR and ARNT [54]. Several studies suggest that curcumin-induced apoptosis is associated with ROS production and/or oxidative stress in transformed cells [55]. For example, curcumin significantly reduced acrylamide-induced ROS production, DNA fragments, micronuclei formation, and cytotoxicity [56]. Curcumin has recently been shown to alter the expression of microRNAs in human pancreatic cancer cells [57,58] where the authors have found eighteen miRNAs that were significantly down-regulated, and eleven miRNAs were up-regulated in pancreatic cancer cells treated with curcumin [58]. Since it is well accepted that miRNA plays important roles in the processes of EMT [7]; therefore, curcumin could be an effective agent for the reversal of EMT via regulating selective miRNAs and by regulating the expression of their respective genes. It has been suggested earlier that the acquisition of EMT or the presence of cancer stem cells is associated with drug resistance, suggesting that selective killing of these cells are required for complete eradication of tumors. To that end, curcumin could become such an agent and in fact a recent study has shown that colon cancer cells that survive the treatment of conventional agents resulted in increased cancer stem cell population, which was effectively killed by curcumin [59]. These and other results suggest that the consumption of curcumin could be a plausible means by which one could prevent carcinogenesis or could revert the carcinogenic process, and thus testifying that further intense investigation in this area is urgently needed.

Among many other natural agents, resveratrol is a phytoalexin present in a wide variety of plant species including grapes, peanuts, and mulberries. High quantities of resveratrol are found in red wine and grape juice. Resveratrol has been shown to elicit beneficial effects on the reduction of oxidative stress. Resveratrol was first noted to be a cancer chemopreventive agent having antioxidant properties [60]. Topical resveratrol significantly reduced 7,12-dimethylbenz(*a*) anthracene (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumors in mice [61]. In the DMBA-TPA mouse skin carcinogenesis model, resveratrol inhibited tumor promotion, possibly due, at least, partly to its antioxidant effects [62]; however the role of resveratrol in TPA-induced ROS production and EMT has not been reported, suggesting that this is a virgin area of research. Recently, resveratrol analog was found to strongly inhibit miRNA-146a promoter-luciferase reporter activity [63]. The miRNA-146a has been shown to be involved in the processes of EMT because miRNA-146a inhibitor augmented the induction of EMT in response to cytokines [64]. These limited studies clearly suggest that resveratrol could be useful for the reversal of EMT phenotype, which could be mediated via the regulation of miRNA-146a, suggesting that further investigation is urgently needed in this area.

Another well known chemopreventive agent comes from green tea because consumption of green tea has been implicated by many studies for the prevention of cancers. Green tea polyphenols (GTP) exhibit biochemical and pharmacological activities including anti-oxidant activities, inhibition of cell proliferation, induction of apoptosis, and modulation of

carcinogen metabolism. It has been reported that GTP rescued the changes in condensed nuclear and apoptotic bodies, attenuated the pro-parkinsonian neurotoxin 6-hydroxydopamine (6-OHDA)-induced early apoptosis, and suppressed accumulation of ROS. In addition, GTP inhibited the auto-oxidation of 6-OHDA and scavenged oxygen free radicals, suggesting that the protective effects of GTP on human neuroblastoma cells are mediated, at least in part, by controlling the ROS pathway [65]. Green tea contains several catechins including epicatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG). Among these, EGCG has been believed to be the most potent for the inhibition of oncogenesis and reduction of oxidative stress. Several studies showed elevation of intracellular ROS production during EGCG-induced apoptosis, suggesting that ROS play a key role in EGCG-induced apoptosis. Furthermore, a combination with arsenic trioxide (As_2O_3) and EGCG significantly enhanced induction of apoptosis via decreased intracellular reduced glutathione levels and increased production of ROS [66]. These limited studies raised more questions than answers to critical questions as to the role of GTP and especially EGCG in ROS homeostasis.

This review article will be incomplete without discussing the role of another interesting agent such as Indole-3-Carbinol (I3C), which is produced from naturally occurring glucosinolates contained in a wide variety of plants including members of the family Cruciferae and particularly members of the genus *Brassica*. I3C is biologically active although it is easily converted *in vivo* to 3, 3'-diindolyl methane (DIM), which is also biologically active. Vegetables of the genus *Brassica* contribute most to our intake of glucosinolates and include all kinds of cabbages, broccoli, cauliflower, and Brussels sprouts. Several studies have shown that DIM induces the release of ROS in cultured tumor cells, suggesting that DIM is a chemical antioxidant. Riby *et al.* reported that hypoxic cells had a basal level of ROS approximately twice that of normoxic cells and the treatment with DIM caused a significantly increase in ROS levels, whereas in normoxic cells DIM caused a smaller increase. These results indicate that DIM is a potent pro-oxidant in hypoxic cells [67]. ROS can cause different biological effects such as autophagy, apoptosis, and necrosis. Autophagy is an intracellular degradation system that delivers cytoplasmic constituents to the lysosome, resulting in the degradation of macromolecules and organelles that can lead to cell survival or cell death in different contexts. Very recently, we found that DIM inhibited H_2O_2 -induced autophagy and at the same time protected cells against oxidative stress in a BRCA1-dependent manner [68]. More importantly, we found that DIM caused the reversal of EMT phenotype in pancreatic GR cell lines through miRNA-200 and let-7 [69], which we believe, could be important for designing novel therapies for the prevention of tumor progression and/or selective killing of EMT-type or cancer stem cells, and thus requires further in-depth studies in the future.

CONCLUSIONS

Although emerging evidence suggest an interrelationship between EMT and cancer stem cells but they are synonymous by no means, hence further research is warranted. In addition, the ROS appear to be involved in various aspects of cancer cell behavior, including cell proliferation, survival, invasion, angiogenesis and metastasis, which are also associated with the acquisition of EMT phenotype that appears to be reminiscent of cancer stem cells. Therefore, targeting ROS would lead to the reversal of EMT characteristics or will selectively kill cancer stem cells that are becoming apparent and necessary for the eradication of cancer. To that end, antioxidant supplements have been used in clinical trials [70,71]; however further in-depth research in this area is urgently needed. Moreover, it is tempting to speculate that we are going to witness rapid developments in assessing the biological significance of chemopreventive agents that could be a safer approach for targeted inactivation of ROS, which in turn will destroy EMT-type cells or cancer stem cells

towards the prevention of tumor progression and/or successful treatment of human malignancies in the future.

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Fig. (1).

A mechanistic diagram showing how ROS could promote EMT progress.