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REVIEW

Ganoderic acid, lanostanoid triterpene: a key player in apoptosis

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Summary Cancer is a multifactorial disease, causing behavioral and metabolic alterations, leading to uncontrolled cell proliferation with collateral weakening of immune system. Crucial balance between cell proliferation and cell death determines the fate of a cell, which might progress towards survival or apoptosis. Apoptosis is a complex, programmed, and highly regulated process causing dramatic morphological and biochemical perturbations in the cellular machinery. *Ganoderma lucidum* is a basidiomycetes, polypore mushroom known for its pharmacological properties in cancer. The major bioconstituents in *G. lucidum* are terpenoids, polysaccharides, and proteins that target cancer-signaling factors like plasma membrane receptors proteins and adapter molecules. Of all constituents, the major terpenoid, i.e. Ganoderic acid is reported to interact with membrane receptors mainly, receptor tyrosine kinase (RTKs). Ganoderic acid interacts and modulates the signaling network in IR, IGFR-1, IGFR-2, VEGFR-1, VEFGR-2, and EGFR in cancer signaling pathways. It primarily targets NF-κB, RAS-MAPK, PI3K/Akt/mTOR, and cell cycle resulting in apoptosis. This review highlights the role of ganoderic acid in apoptosis and modulations of various signaling proteins in cancer.

Keywords *Ganoderma lucidum* · Ganoderic acid · Apoptosis · Cancer · Cell cycle

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Introduction

The impaired regulation of gene networks causes altered behavior and metabolism of the cell and disturbs the signaling pathway, engendering cancer or cancer-like conditions. An alarming increase in the number of cancer patients and commercialization of several drugs for other diseases, fuels the increased hunt of new restorative drugs in cancer signaling [1, 2]. Natural products and their derivates provide a wide range of compounds that work on a target specific approach for drug designing [3, 4]. Nevertheless, the complex gene networks involved in cancer signaling are a hurdle in targetting specific genes. Therefore, apoptosis, which is cell's own process for a pre-programmed death, is a better alternative target in cancer therapeutics. Major signaling pathways in cancer are regulated by NF-κB, RAS-MAPK, PI3K/Akt/mTOR, and cell cycle mediated apoptosis [5]. *Ganoderma lucidum*, a polypore fungus, reportedly, possesses pharmacological properties and is involved in cancer cell death by apoptosis.

Background of apoptosis

Cell survival depends up on the critical balance between cell division and cell death. Carl Vogt first highlighted the concept of natural cell death during developmental studies in toads, in 1824. Later in 1885, Flemming depicted hallmarks of apoptosis viz. cell shrinkage, apoptotic body formation, nuclear fragmentation, in a pictorial representation. In 1965, term shrinkage necrosis was coined by Kerr, who also proposed the role of lysosome enzyme in necrosis [6]. In 1942, Kerr et al. termed controlled cell deletion as "apoptosis" and marked it important for maintaining animal cell populations [7]. Later in 1980's, DNA ladder formation and the role of Bcl-2 was also observed in apoptosis. In 1990's, further development in the field of apoptosis described the role of bcl-2, p53, MYC, BAX, BAK, and antisense RNA in apoptosis [8]. In 2002, Bob Horvitz received Nobel prize in Physiology or Medicine for his pioneering work in the field of apoptosis using *Caenorhabditis elegans* as model.

Cellular changes during apoptosis

In apoptosis, other than diseased cells, cells which escape the immune response are also phagocytized by triggering activation of caspases [9]. Caspases are cysteine proteases, which target cell death by restricted proteolysis without harming the neighboring cells. After cell death, cell debris get phagocytized and new cells are appointed to the site within a few hours. The distinctive features of nuclear condensation and its fragmentation serve as a marker, in the absence of other specific markers, of apoptosis and are characterized by hydrolysis of nuclear content in to multiples of 200 base pair fragments. This is endonuclease mediated process, cleaving the inter-nucleosomal site ensuring in condensation of chromatin [10]. Besides nuclear fragmentation, endoplasmic reticulum, Golgi bodies, and mitochondria also undergo fragmentation with release of various proteins from mitochondrial outermembrane. These released proteins initiate the cascade of caspase activation, which cleaves and directs the downstream events leading to apoptosis. Activation of caspases distorts the cytoskeleton and cleaves the actin, myosin, gelsolin, filamin, tubulin and intermediate filaments such as vimentin, keratin, nuclear lamina. Nuclear lamina and nuclear envelope disintegration (Fig. 1) is induced by proteolysis of lamins A, B [11]. Importantly, ROCK1, actin filament is marked vital in the structural integrity of nuclear envelope, and inhibition of this protein stimulates the fragmentation events [12].

Apoptotic cells disrupt the extracellular matrix (ECM) with the help of caspases and don't pass the lethal signals to healthy neighboring cells. The detachment of apoptotic cells from ECM is instigated by proteolysis of focal adhesion proteins, leading to cell-matrix breakdown [13]. Also, the series of fragmentation events render chromatin more prone to phagocytosis and avoid the activation of immune system [14]. In nuclease deficient mice, which lacks caspase activating DNAse (CAD), a surge in immune components and other downstream signaling molecules is observed that initiates the progression of autoimmune state [15]. In healthy growing cells, CAD exists in association with inhibitors of caspases activating of DNase (ICAD), which repress its activation. In apoptotic cells, ICAD gets cleaved by caspases ensuing in activation of CAD and thus, chromatin fragmentation [16]. Other organelles such as Golgi bodies, endoplasmic reticulum also undergo caspase-mediated fragmentation by cleaving Golgistacking protein GRASP65, golgin160, p115, syntaxin5 and giantin causing breakdown of Golgi [17]. In apoptosis, ER is also remodeled and forms chromatin anapoptotic bleb (Fig. 1).

Caspases cause destructive morphological changes in mitochondria by proteolysis of the p75 subunit of complex I in electron transport chain. This is caused by series of events linked with a decrease in the level of ATP and enhanced reactive oxygen species (ROS) production, which disrupts the integrity of p75 subunit [18]. Caspases proteolytically cleave various transcription and translational factors like AP2a, BTF3, NFATc1, NFATc2, NFκBp65, SP1, eIF2a, eIF3, eIF4B, eIF4E, eIF4G and eIF4H [19].

The most striking feature of apoptosis is definitely an adequate disposal of apoptotic cell debris via phagocytosis. In phagocytosis, minimum production of pro-inflammatory cytokines such as tumor necrosis alpha (TNF-α) is done, resulting in the activation of MHC, and thereby immunological events resulting in effectiveS disposal [20]. The membrane blebbing and its pinching off, lead to the disruption of cytoskeleton of the cell and generates apoptotic bodies [21]. Another hallmark of apoptosis is a caspase-dependent translocation of inner plasma membrane phosphatidylserine to the outer side of the membrane, where it undergoes phagocytosis. During phagocytosis, peptide induced apoptotic cells enter MHC-I antigen presenting pathway and their engulfment is derived by caspases [22].

Terpenoids in cancer

Numerous drugs and therapies are designed to target various signaling proteins involved in cancer. Drugs are designed specifically to target particular protein, but cancer usually involves a network of genes and is apprehensive to control. As a result, a continuous hunt of new therapeutic drugs, become need of the hour. Natural products are a rich source of bioactive molecules and are efficient in context to target specificity and dose or time dependent responses. The cytotoxic

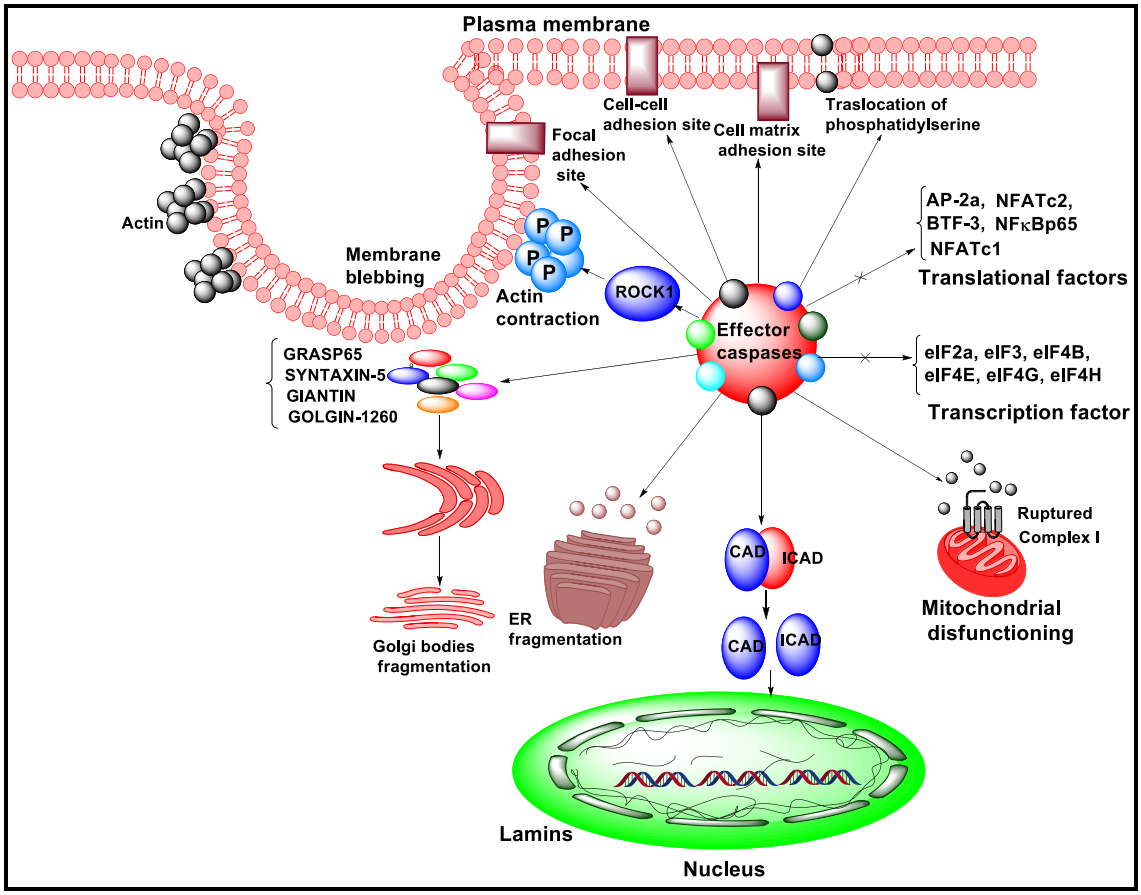

Fig. 1 Caspase mediated cellular changes during the process of apoptosis. The effector caspases cleave various physiological substrates in the cell. In healthy cells, CAD remains inactivated by ICAD, but during apoptosis it gets cleaved, causing fragmentation of genetic material. Caspases target nuclear lamins engendering proteolysis of the nuclear envelope and nuclear fragmentation. Similarly, structural integrity of Golgi apparatus and endoplasmic reticulum get distorted with the caspases action. ROCK1 phosphorylates and cleaves the contraction of actin generating bleb in the plasma membrane. Caspases translocate phosphatidylserine from inner plasma membrane to outer membrane where it undergoes phagocytosis

nature of existing drugs, demands alternative drugs with minimal side effects and more target specificity [23]. Terpenes, with isoprene as a basic monomeric unit, constitute one of the major class of natural products, known for its therapeutic effects. The bioactive properties of terpenes range from anti-inflammatory, anti-tumorigenic to hypolipidemic activity [24]. The structural classification of terpenoids is based on the basic number of monomeric isoprene units used, and are classified in to monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}). Among various natural products, a basidiomycetes fungi, *Ganoderma lucidum* commonly considered a medicinal herb, has been used as a medicine since ancient times [25]. The name *Ganoderma lucidum* is derived from Latin word *lucidus* meaning “shiny” or “brilliant”, owing to its smooth surface. This polypore fungus belongs to family Ganodermataceae and possesses double walled basidiospore with a shiny surface and thick-walled pilocystidia in extracellular melanin matrix [26]. Different species of *Ganoderma* are present with almost similar morphological and identification features, which make its identification cumbersome for taxonomists. The common morphological and anatomical features encompass shape and size of basidiocarp, colour of pore surface and context, pore size, colour of pileus and stipe, size and the shape of basidiospore [27]. The medicinal values stimulate the immunity and override the physiological nuisance it creates to its host plants. Most affected host plants are *Pinus, Dalbergia, Artocarpus, Cedrus, Melia, Quercus,* and *Populus,* which undergo wilting, impairment of vascular system, and even mortality in severe cases [28]. Previously our research group demonstrated the anticancer potential of *G. lucidum* grown under abiotic stress conditions. In this study, *G. lucidum*-host plant interaction was characterized with the high level of phytochemicals to adapt and survive in the incompatible abiotic conditions. Interestingly, *Ganoderma* grown on host plant *Azadirachta* has the highest content of phytochemicals

with effective anticancer potential [29]. Among various triterpenes present in *G. lucidum*, ganoderic acids are found and explored in cancer signaling.

Biosynthesis of terpenes (mevalonate pathway)

Fruiting bodies, mycelia and spore found in *G. lucidum* are the primary source of ganoderic acids (GAs) production. GAs are synthesized by generation of isoprenoid like that in case of terpenes and follow the mevalonate pathway [30]. In this pathway, acetyl-coenzyme A gets converted into isopentenyl pyrophosphate (IPP). Prenyltransferases further synthesize the geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP) from IPP in a series of steps, constituting oxidation, isomerization, reduction, and conjugation. These chemical changes contribute to the unique basic skeleton of terpenoid family (Fig. 2). An alternative pathway is non-mevalonate pathway also known as methylerythritol 4-phosphate (MEP) wherein the first intermediate is deoxyxylulose (DX) 5-phosphate. DX is obtained after a series of reactions involving condensation of pyruvate and D-glyceraldehyde 3-phosphate that undergoes rearrangement and forms DX. 1-hydroxy-2-methyl-2-(E)-butenyl 4diphosphate converts 2-C-Methyl-D-erythritol, 2,4-diphosphate into IPP and DMAPP [31].

Bioconstituents of *Ganoderma lucidum*

The mushrooms are mainly composed of water (approximately 90%), protein, fat, and carbohydrate along with traces of fiber, ash, vitamins, and mineral [32]. *G. lucidum* contains terpenoids, polysaccharides, and proteins including the small amount of amino acids and vitamin [33, 34]. Amongst terpenes, triterpenes, mainly ganoderic acids, are the major class in *G. lucidum,* which was explored more due its potential therapeutic value. There are more than one hundred isoforms of ganoderic acids isolated and characterized from fruiting bodies, spores, and mycelia of *Ganoderma* species [35]. Different extracts of *G. lucidum* were prepared and exhibited varying degrees of anticancer potential in different cell lines [36]. These variations in anti-cancer property may be because of the solubility of extracts, altered physical parameters during isolation or purification of the compound or/and different physiological states of different cancer cells. Alcoholic extract of *G. lucidum* inhibited cell proliferation in a dose and time-

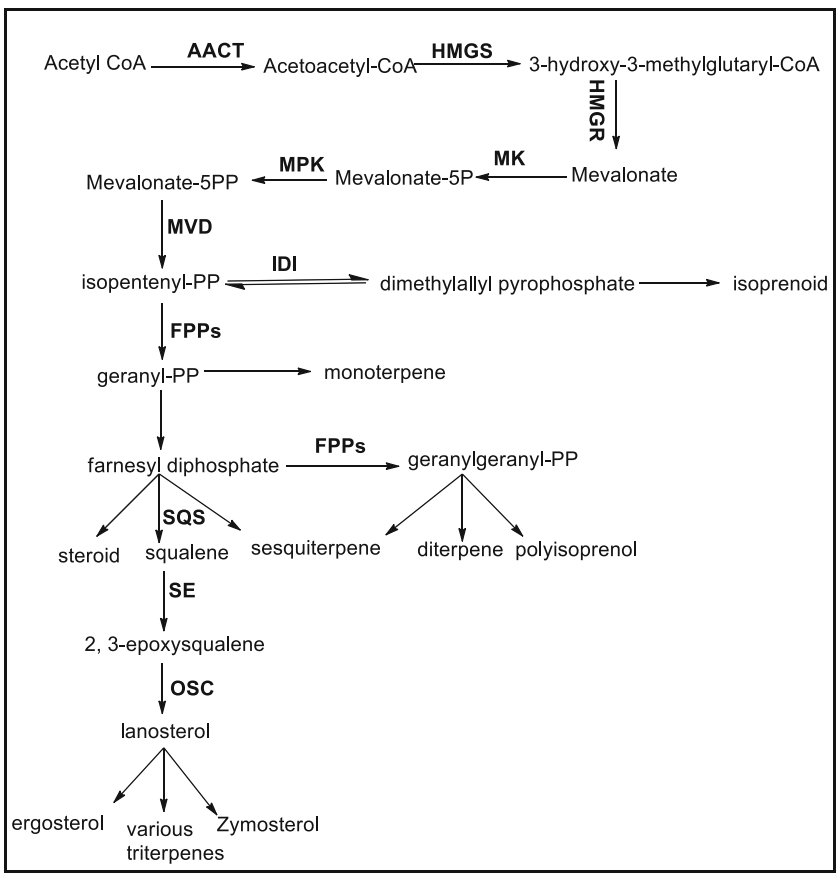

Fig. 2 Schematic representation of mevalonic acid pathway. AACT; acetyl-CoA acetyltransferase, HMGS; 3-hydroxy-3- methyl glutaryl-CoA synthase, HMGR; 3-hydroxy-3-methyl glutaryl-CoA reductase, MK; mevalonate kinase, MPK; phosphomevalonate kinase, MVD; pyro phosphomevalonate decarboxylase, IDI; isopentenyl diphosphate isomerase, FPPs; farnesyl diphosphate synthase, SQS; squalene synthase squalene, SE; monooxygenase, OSC; 2, 3-oxi do squalene-lanosterol cyclase,, FPPs; geranylgeranyl-PP synthase

dependent manner in MCF-7 cells, which was attributed to p21/Waf1 and cyclin D1 that also induce apoptosis through upregulation of proapoptotic bax protein. Extracellularly, triterpenes stimulate expressions of IL-6 and TNF-α, arrest cell cycle at the G2/M phase, which are also involved in triggering apoptosis. Apoptosis is associated with decreased levels of Bcl-2 and pro-caspase 9 with consequent increase in caspase 9 in A549 cells [37]. Likewise, ethanolic extract of sporoderm-broken spores of *Ganoderma lucidum* (SBGS) are reported to arrest cell cycle in lung cancer. SBGS arrest cell cycle in G2/M phase and decrease the level of cyclin B, cdc2, Bcl-2 and Bcl-xl. Furthermore, oral administration of SBGS in mice suppressed the activation of Akt, mTOR, S6 kinase and 4E–BP1 in tumor cells and modulated the downstream signaling events leading to various physiological processes [38]. Triterpenes isolated from fruiting bodies rich fraction, WEES-G6, inhibited the growth of human hepatoma Huh-7 cells with simultaneous decreased level of PKC by phosphorylion of JNK and p38 MAP kinases [39]. The combinatorial system used to synthesize Khz by fusing mycelia of *G. lucidum* and *Polyporus umbellatus,* exhibited proapoptotic potential. Khz arrests cell cycle at G1 phase and reduced MMPs and Bcl-2 but enhanced the level of ROS production along with p53 and pro-apoptotic proteins. Thus, Khz shows anti-proliferative and pro-apoptotic effects and behave as a chemotherapeutic agent [40] (Table 1, Fig. 3).

Role of ganoderic acid in apoptosis

Apoptosis is initiated by various stimuli such as external growth factors, hormones, cytokines and neurotransmitters in response to which cell membrane receptors get phosphorylated and activate the downstream signaling molecules. Different types of receptors present in the plasma membrane are divided on the basis of tertiary structure or topology of the membrane. The G protein–coupled receptors (GPCRs) and receptor tyrosine kinase (RTKs) are a major class of membrane receptors involved in signal transduction pathway. GPCRs are the seven-transmembrane domain receptors with cAMP and phosphatidylinositol signaling pathway while RTKs are transmembrane glycoproteins which up on activation get stabilized by receptor dimerization [56]. RTKs family is classified on the basis of recognition by ligands and their structure, and is critically involved in various processes leading to cell proliferation, differentiation, and migration. RTKs family includes signaling factors like insulin growth factor (IGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and nerve growth factor (NGF) [57]. Monomeric as well as multimeric subunits are found in RTKs, which get activated through phosphorylation of phosphotyrosine-binding (PTB) and Src homology 2 (SH2) domains [23]. In our previous study, different isoforms of ganoderic acid in RTKs were evaluated for mechanistic binding with IR, IGFR, VEGR1, VEGFR2, ER receptors. The study disclosed the nature of binding between proteins and receptors revealing the lipophilic, hydrogen bonding, and pi-pi stacking interactions. The binding patterns differ among members of RTKs and vary with side chain or group attached to the basic lanosterol moiety [49].

Jiang et al. reported the potential of GA-A and GA-H in breast cancer where these isoforms suppress cell proliferation, metastasis, and adhesion with a decrease in Cdk4 and urokinase-type plasminogen activator (uPA) expression in AP-1 and NF-κB nuclear transcription factors [48]. Furthermore, mechanistic binding involved in NF-κB was highlighted and showed the role of different residues in GA-A, GA-H, and GA-F in apoptosis [50]. Ganoderic acid A targets STAT3 and inhibits cell proliferation, viability, and ROS in a dose-dependent manner in PC-3 cells [51]. Similarly, GA-A targets Wnt protein and affects the cell proliferation, viability, and ROS in a dose-dependent manner in

Table 1 Different ganoderic acids and associated proteins in the process of apoptosis

Ganoderic acid	Process involved in apoptosis	References
Ganoderic acid	Cell proliferation, viability, ROS, TNF	[41]
GA-Mf	bax/bcl2, cell cycle in S phase, caspases	[42]
Ganoderic acid T	P53, uPA, (MMP-2/9), inducing nitric oxide synthase (iNOS/NOS2), G1cell cycle arrest	[43–47]
GA-S	bax/bcl2, cell cycle in S phase, caspases	[42]
GA-A	AP-1, NF-κB, IR, IGFR, VEGR1, VEGFR2, ER	[48–51]
GA-DM	Cell proliferation, colony formation, G1 cell cycle arrest, CDK2, CDK6, cycle D1, p-Rb, c-Myc, DNAfragmentation, PARP	[52]
GA-Me	MMP2/9, arrest cell cycle at G1, bcl-2/bax, mitochondrial membrane potential	[46 ,47, 53]
GA-X	Topoisomerases, ERK, JNK, MAPKs, Bcl-xL, MMPs($\Delta\psi_m$), cytochrome-c	[54]
GA-D, GA-T	14–3-3ζ protein, annexin A5, aminopeptidase B, cell cycle at G2/M phase, p53, IL-2, IFN-γ, NF-κB, bax, bcl-2, caspases	[43, 55]

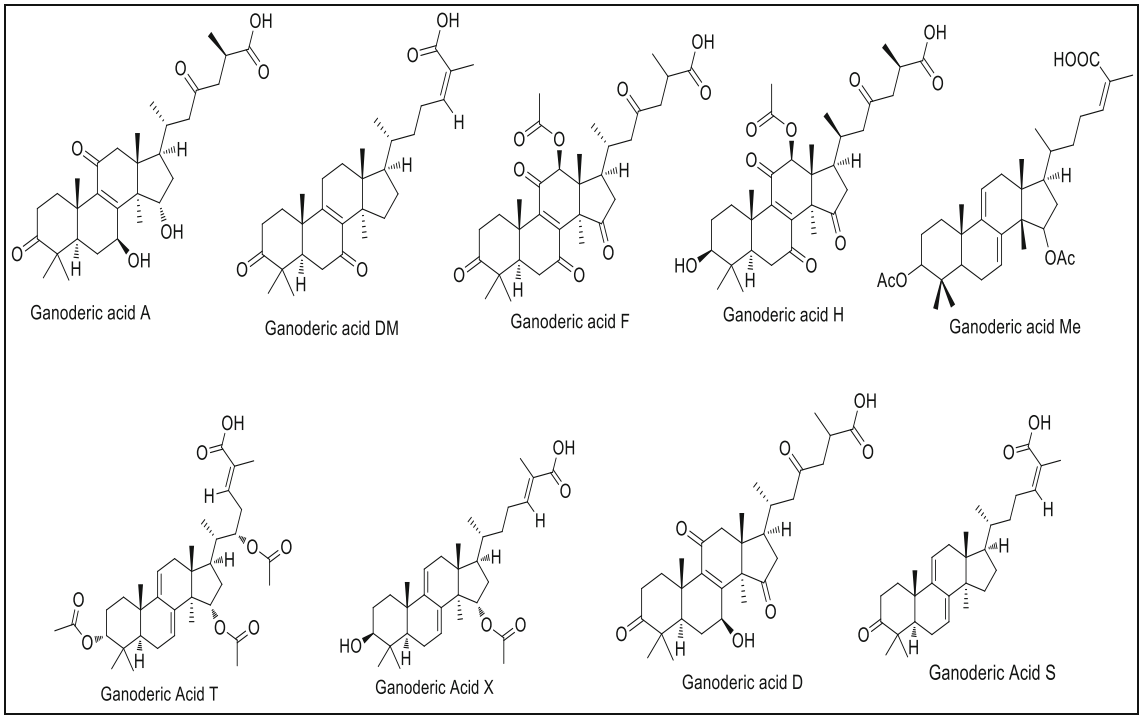

Fig. 3 The depiction of the chemical structure of various isoforms of ganoderic acids

pancreatic cancer cells [24]. GA-X target the apoptosis via mitochondrial pathway in association with topoisomerases, ERK, JNK, mitogen-activated protein kinases, bcl-xL resulting in release of cytochrome c [54]. Tang and his team isolated GA-Me from the methanolic extract of *G. lucidum,* which arrests cell cycle in G1 phase and checks intrinsic mitochondrial-mediated apoptosis in athymic mice [43]. In a similar study, GA-Me is reported to target apoptosis and arrest cell cycle in G1 phase with decreased level of bcl-2/bax by p53, resulting in a reduction in mitochondrial membrane potential in colon cancer cells [46]. Similarly, GA-Me in 95-D cells induced cell homotypic aggregation with inhibition of MMP2/9 expression [47]. In the apoptosis process, GA-D and GA-T bind to the 14–3-3ζ protein, annexin A5, aminopeptidase B, arrest the cell cycle inG2/M phase which causes precipitation of p53, IL-2, IFN-γ, NF-κB, bax, and bcl-2 in programmed cell death in HeLa cells [55, 58, 59]. In vitro and in vivo studies suggested the antiproliferative nature of GA-T in colon and lung cancer by modulations in uPA, (MMP-2/9), and nitric oxide synthase (iNOS/NOS2) [43–47]. Likewise, GA-S and GA-Mf induced mitochondria-mediated apoptosis in HeLa cells by targeting mitochondrial membrane potential and arrested the cell cycle in S phase with modulations in bax/bcl2 ratio and consequent activation of caspases and cytochrome c release [42]. Similarly, GA-DM inhibited cell proliferation, colony formation, G1 cell cycle arrest with decreased level of CDK2, CDK6, cycle D1, p-Rb, c-Myc and assiciated induction in DNA fragmentation and cleavage of PARP in MCF-7 cells [52]. Furthermore, ganoderic acid modulates the functioning of nuclear factor erythroid 2–related factor 2 in cancer signaling which is responsible for activating various antioxidant enzymes [60]. This suggests that ganoderic acid behaves as an important signaling modulator in cancer with great potential in cancer therapeutics.

Side effects

G. lucidum has a diverse roles in apoptosis highlighting its involvement in signal transduction in cancer. Spore powder of *G. lucidum* proves effective against fatigue in breast cancer in endocrine therapy. Repeated follow ups reveal that patients feel less anxious and depressed, without any side effect [61]. Administration of *G. lucidum* does not cause any lethal effect, but dosage in the form of spore capsule, freeze dried powder, tablets, soup, and syrup may cause some adverse effect depending on dose. The oral dosages (1.5 g/day) may cause mild effect causing sleepiness, thirst, rashes, bloating, and urination [62]. No harmful effect in any body organ responsible for growth, was observed while taking an alcoholic extract of *G. lucidum* (1.2 and 12 g/kg daily) for 30 days. Interestingly, drug combination with lithium, perphenazine, and trihexyphenidyl with *Ganoderma* during schizophrenia treatment have no side effects on patients [63]. However, Kawagishi et al. reported that *G. lucidum* interacts with aspirin or warfarin drugs therapies resulting in several side effects [64]. Further investigations are suggested to rule out the possibility of lethal side effects, if any.

Conclusion

Ganoderic acid, a triterpene, from polypore fungus targets apoptosis via either intracellular or extracellular pathway, by regulating various proteins and adapter molecule in cancer signaling. Various isoforms of ganoderic acid regulate the functioning of plasma membrane receptors mainly in RTKs family and control the downstream signaling in cancer. The basic lanosterol scaffold in the ganoderic acid remains the same, while the side chain or functional units vary and decide the function of particular ganoderic acid. Ganoderic acid modulates NF-κB, PI3K/Akt/mTOR, cell cycle, mitochondrial membrane potential, and cytochrome c leading to apoptosis in cancer cells. Future prospective includes a computational screening of particular ganoderic acid with quantitative structure–activity relationship (QSAR) to target specific genes in the apoptosis. To isolate different and new terpenes in *G. lucidum,* further investigations are required which might find the bridge the gap between drugs and different diseases.

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Compliance with ethical standards

Conflict of interest None.

Ethical approval This article does not required any ethical approval.

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