Chapter

Cell Death Mechanisms of the Promising Anticancer Compound Gallotannin

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Abstract

The polyphenolic hydrolyzable tannin, gallotannin (GT), also known as tannic acid, possesses interesting anticarcinogenic properties. An evidence from experimental studies suggests that GT is effective against multiple cancer types. Gallotannin has been shown to induce programmed cell death in a wide variety of cancers including colon, breast, prostate, and liver, among others. Apoptosis, cellular senescence, autophagy, and necrosis are the main mechanisms by which GT can suppress cancer progression. In addition, GT is a potent inhibitor of many proliferation pathways. Herein, this chapter provides a summary of our current knowledge about GT’s programmed cell death mechanisms against cancer.

Keywords: programmed cell death, apoptosis, autophagy, necroptosis, cellular senescence, polyphenols, gallotannin, cancer

1. Introduction

Over the past decade, cancer was considered as the major public health concern and one of the leading causes of death worldwide with 9.6 million cancer deaths reported in 2018. The main recurrent cancers are lung, colorectal, stomach, liver, prostate, and breast. Various external (tobacco, radiation, infections, nutrition) and internal (mutations, hormones, and immune conditions) factors contribute to cancer incidence. Described as a group of diseases characterized by uncontrolled proliferation and growth of abnormal cells, cancer is mainly characterized by its resistance to cell death and sustained activation of proliferation pathways. Throughout life, there is a balance between cell death and cell proliferation [1]. Any imbalance favoring one process over the other would contribute to the development of significant disorders such as autoimmune diseases, cancer, AIDS, neurodegenerative disorders, myocardial infarctions, atherosclerosis, and insulin-dependent diabetes. Furthermore, cell death can be divided into two forms: regulated and accidental. Unlike regulated cell death which relies on the activation of signal transduction cascades and can be modulated pharmacologically or genetically, accidental cell death occurs immediately in response to physical (high pressure, osmotic forces, temperatures, etc.), chemical (extreme pH variations, etc.), or mechanical (shear forces, etc.) cues that occur in an uncontrollable form [2]. Besides apoptosis, there are many forms of programmed cell death, namely, necroptosis, cellular senescence, autophagy, slow cell death, and paraptosis [3].
One third of all cancers can be prevented by a healthy lifestyle which includes an appropriate and balanced nutrition [4]. There is a growing interest in using dietary compounds as preventive and therapeutic agents in cancer due to their relative safety, their immediate action on target tissues, and their specific action against cancer cells [5]. The protective effects of dietary compounds are a consequence of different modes of action such as their ability to neutralize carcinogens, hamper the transcription of oncogenes, activate detoxifying enzymes, and trigger cell death in mutated and cancerous cells.

Polyphenols are a group of phytochemicals that when consumed decrease the risk of chronic diseases especially cancer. They are effective in treating solid tumors by inducing a cohort of effects such as cell cycle arrest and cell death [6]. The polyphenolic hydrolyzable tannin, galloにつき, and penta-1, 2, 3, 4, 6-O-galloyl--
beta-D-glucose (PGG), a precursor of GT, have been shown to exert various biological effects ranging from anti-inflammatory to anticancer effects in various tumor cells [7]. Thus, current research aims at developing therapeutic approaches that would benefit from the interconnected matrix of signaling pathways and the ability of natural compounds to induce programmed cell death processes that could prevent and restrain tumor development.

2. Programmed cell death mechanisms

2.1 Apoptosis

Each day, our body eliminates, via apoptosis, billions of unwanted cells. In addition to being essential for development and homeostasis, apoptosis is the major mechanism of programmed cell death. During apoptosis, apoptotic cells undergo characteristic changes in cell morphology, including shrinkage of nuclei, nuclear chromatin condensation, cytoplasmic shrinkage, dilated endoplasmic reticulum, membrane blebbing, and the exposure of specific phagocytic signaling molecules on the cell surface [8]. The contents of the cell are engulfed in apoptotic bodies which are then recognized by the phagocytic cells and digested in lysosomes. In most cells, apoptosis leads to the activation of caspases which mediates the auto destruction of the cell. Caspases, a family of cysteine proteases, exist as inactive precursors (pro-caspases) and are activated upon cleavage. The C-terminal side of a four amino acid motif, X-X-X-Asp (where X can be any amino acid) is the preferred cleavage site for the known caspases. Activated caspases in turn cleave various intracellular and cytoplasmic membrane substrates, leading to cellular disintegration [9].

2.1.1 Apoptotic pathways

In the mammalian system, two major pathways lead to apoptosis: the intrinsic pathway which involves the mitochondria and the extrinsic pathway which is initiated by death receptors [10]. The intrinsic pathway of apoptosis is mediated by the Bcl-2 family protein (also known as mitochondrial or stress pathway). Bcl-2 family, composed of both anti-apoptotic and pro-apoptotic proteins, is generally divided into three subgroups based on their roles in apoptosis and the BH regions they share: one anti-apoptotic group and two pro-apoptotic groups. The anti-apoptotic group includes Bcl-2, Bcl-xL, Bcl-w, Bcl-B, A1, and Mcl-1, which share three or four BH regions. The pro-apoptotic Bcl-2 family members include Bax, Bak, Bcl-xs, Bok, and Bcl-GL, which have two or three BH domains. Another pro-apoptotic group contains the BH3-only proteins, including Bad, Bid, Bim, Bik, Noxa, Puma, Bcl-Gs, Blik, Bmf, and Hrk, which share only the BH3 domain. A key
event of the intrinsic pathway is the mitochondrial outer membrane permeabilization (MOMP) process, which is considered the point-of-no return in apoptosis induction. Normally, the anti-apoptotic members of the Bcl2 family prevent MOMP [11]. In response to stress stimuli such as oncogenes, direct DNA damage, oxidative stress, and starvation, two pro-apoptotic proteins, Bax and Bak, become activated by BH3-only proteins that serve as sensors for apoptotic stimuli. Once activated, Bax and Bak permeabilize the outer membrane of mitochondria, causing the release of pro-apoptotic factors such as cytochrome c. In the cytosol, cytochrome c binds to monomeric apoptotic protease activating factor-1 (APAF-1) at its WD40 domain and induces a conformational change in APAF-1 promoting APAF-1 oligomerization and initiating the formation of the apoptosome. APAF-1 then binds to pro-caspase 9 resulting in its auto-cleavage and release of active caspase 9. Active caspase 9 then cleaves the effector caspases, such as caspase 3 and caspase 7, resulting in their activation and promoting the cell death process. The extrinsic pathway or death receptor pathway involves the binding of ligands to cell surface “death receptors” which in turn initiate the caspase cascade. Death receptors, located on the cell membrane, are members of the TNFR family and are characterized by the presence of a death domain (DD) that plays a crucial role in apoptotic signal transduction [12]. The best characterized ligands and their corresponding death receptors include TNF-α/TNF-R1, FasL/FasR, APO3L/DR3, TRAIL/TRAIL-R1, TRAIL/TRAIL-R2, and TRADD/DR6. The binding of death ligands results in the oligomerization and the activation of the death receptors. Oligomerization of the receptors is followed by binding of specific adapter proteins (FADD, TRADD) to their receptor, which in turn leads to the activation of the caspase signaling pathway. FADD binds to pro-caspase 8 through its dead effector domain (DED) allowing the formation of DISC, the death-inducing signaling complex, and the autocatalytic activation of pro-caspase-8. Active caspase-8 executes the apoptotic process through direct cleavage and activation of effector caspases (caspases 3, 6, and 7) [13].

2.1.2 Apoptosis and cancer

The evasion of apoptosis is one of the prominent hallmarks of cancer cells. This is the result of mutations in apoptosis-related genes. The Bcl-2 family members, Fas, p53, and c-Myc are the common mutated genes in cancer. Overall, malignant cells, in different kinds of cancers, have an anti-apoptotic phenotype with low level of pro-apoptotic proteins such as Bax and high level of anti-apoptotic proteins such as Bcl-2 and Bcl-xL. The tumor suppressor p53 is mutated in most cancers including colorectal carcinoma, brain and lung cancer, mammary carcinoma, and skin and bladder carcinomas. The overexpression of inhibitor apoptosis proteins (IAP) and downregulation of surface death receptors (CD95, DR4, and DR5) were also detected in cancer cells [14].

2.2 Cellular senescence

Senescence is a normal process caused by telomere shortening after successive cell divisions of normal somatic cells. This process irreversibly halts the cell from proliferation. In addition to being a normal process, senescence can be activated in response to oncogenic activation, oxidative stress, and DNA damage. Morphologically, senescent cells can be distinguished by their enlarged, flattened, and granular morphology; nuclear enlargement; and altered chromatin structure [15]. Biochemically, cellular senescence is characterized by an enhanced β-galactosidase activity, inhibition of cyclin-dependent kinases (CDKs), the absence of proliferation markers, and the presence of senescence-associated heterochromatin foci (SAHF) [2].
2.2.1 Pathways of cellular senescence

The molecular pathways of senescent cells are not unique but differ between cells from different species and among different cell types from the same species. The heterogeneous pathways of senescence, however, meet at p53 and p-Rb [16]. In the p53-p21 pathway, p53 is the important player of the senescence response. The expression of the phosphorylated form of p53 increases in senescent cells leading to an increase in its transcriptional activity. P53 is activated in response to shortened telomeres that activate a DNA damage cascade through ATM/ATR and Chk1/Chk2 resulting in G1 phase arrest. P53 is also activated in response to DNA damage, oxidative stress, and activation of Ras oncogene leading to telomere-independent premature senescence. One of the most important targets of p53 is p21 which can also be expressed in a p53-independent manner [17]. Some cell types have been found to undergo senescence upon overexpressing p21 and escape senescence upon its deletion. Mouse embryonic fibroblasts lacking p21 undergo senescence suggesting that senescence can occur in a p21-independent manner in these rodent cells [18]. In the p16-pRb pathway, p53 and p-Rb are thought to act simultaneously to achieve senescence because their concomitant inactivation is needed to terminate the senescence response in human cells. The phosphorylation state of Rb controls the progression through the cell cycle. When phosphorylated by cyclin-dependent kinases, p-Rb liberates E2F transcription factor that transcribes target genes responsible for DNA replication and progression through the cell cycle. Inhibition of CDKs by cyclin-dependent kinase inhibitors prevents the phosphorylation of Rb. When hypophosphorylated, Rb binds E2F, thus preventing the transcription of E2F target genes [19]. CDKIs belong to two families: the CIP/KIP family including p21, p27, and p57 and the INK4 family including p15, p16, p18, and p19. P16 expression is found to be high in senescent cells driven to cell cycle arrest by stressful stimuli such as DNA damage, oxidative stress, and oncogenic Ras activation. Both p53-p21 and p16-pRb senescent pathways converge on inhibiting the phosphorylation of Rb [20]. In some cases, senescence is hindered by the inhibition of either p53 or p-Rb which suggests a sole p53 and p-Rb signaling pathway. In other cases, inhibiting senescence requires the inactivation of both p53 and p-Rb supporting the existence of two simultaneous pathways. This variation in the molecular mechanisms of the senescence signaling pathway depends on several factors including p16 expression, the type of cell line, culture conditions, and the amount of stress [21].

2.2.2 Cellular senescence and cancer

Senescence represents a fail-safe mechanism that guards against oncogenic transformation [22]. Cancer arises after a normal cell accumulates several mutations that are inherited with each replicative cycle. Senescence helps limit the accumulation of mutations by restricting the replicative ability of normal cells, thereby preventing cancer development [23]. Furthermore, it has been shown that initial oncogenic events will lead to senescence, and at that point the senescence-associated secretory phenotype will induce immune clearance limiting early tumor growth [24]. But many cancerous cells acquire indefinite proliferation by escaping senescence through different mechanisms that inhibit telomere shortening such as triggering telomerases or increasing telomere length by homologous recombination or inhibiting tumor suppressors. In fact, various tumor suppressors and oncogenes have been shown to regulate senescence in normal cells, and senescence bypass appears to be an important step in the development of cancer [25]. For instance, inhibiting BRAF-induced senescence by the loss of the tumor suppressor PTEN will lead to melanoma progression [18]. Thus, evading senescence is an important step...
toward full malignancy and metastasis. Tumor cells may escape senescence indirectly by acquiring mutations that affect senescence-related proteins such as the tumor suppressor proteins p53 and p-Rb which are frequently mutated in cancer [26]. Furthermore, many studies detected senescent cells in premalignant mice and human tumors and not in the malignant tumors. This senescence response was thought to have an antitumorigenic role in cancer-predisposed tissues [27].

Although most cancer cells bypass oncogene-induced senescence, various studies described the anticancer ability of chemotherapeutic drugs to induce senescence in those cancer cells, an event which was termed as therapy-induced senescence.

2.3 Autophagy

In response to starvation, hypoxic conditions and high temperatures, and DNA and organelle damage, the autophagy cell death process is activated. Double-membrane cytoplasmic vesicles called autophagosomes engulf cytoplasmic organelles and fuse to lysosomes to form autolysosomes when the cellular components are digested [28].

2.3.1 Pathways of autophagy

Autophagy is driven by Atgs proteins and is regulated by PI3 kinase types I and III. PI3K type 1 inhibits autophagy through PDK1 and AKT which regulate mTOR. Atg6 (Beclin1) is part of PI3K type 3 complex which promotes the nucleation of autophagic vesicles. In resting conditions, mTOR phosphorylates and inactivates ULK1 (Atg1). Metabolic stress activates AMPK which inhibits mTOR activity and activates ULK1 leading for the activation of the autophagy function of PI3K type 3 through Beclin1 phosphorylation [29]. The proteins Atg1 and Atg13 allow the membrane isolation. Elongation is then mediated by Atg10, Atg5, and ATG12. The recruitment of the cytoplasmic protein LC3 by Atg7, Atg4, and Atg3 to the nascent autophagosome is necessary for the expansion and fusion events. The formation of autolysosomes is not only sufficient for autophagic cell death; additional death signals are still needed. Enhanced expression of c-Jun N-terminal kinase (JNK) generates such signals and rapidly induces autophagy cell death [30].

2.3.2 Autophagy and cancer

Decreased rate of autophagic activity is related to tumorigenesis, and autophagic cell death does not occur in most cancer cells. Beclin1 is downregulated in many cancer types including prostate, breast, and ovarian cancer [31]. Mutations of Atg5 and LC3 (microtubule-associated protein 1 light chain 3B) promote myeloma and glioblastoma, respectively [32]. JNK activation is significantly decreased in cancer cells [33].

2.4 Necrosis

Although most scientists considered necrosis as accidental cell death and an uncontrolled process, several studies showed that some forms of necrosis are programmed [34]. Necroptosis is controlled by receptor interacting protein (RIP) kinases, NADPH oxidases, poly(ADP-ribose) polymerase-1 (PARP1), and calpains [35]. During necroptosis, the disruption of the cellular membrane integrity leads to the release of the intracellular materials in the extracellular environment which induce a local inflammatory response. These perturbations are detected by specific death receptors such as FAS and TNFR1 or pathogen recognition receptors (PRRs).
such as TLR3 and TLR4 [2]. The activation of receptors allows the activation of RIPK1 which in turn activates RIPK3. The signaling complex formed by RIPK1 and RIPK3 is known as necrosome and leads to the phosphorylation of mixed lineage kinase domain-like pseudokinase (MLKL). The activation of MLKL results in the formation of MLKL oligomers that upon translocation to the plasma membrane trigger its permeabilization [30].

2.4.1 Necroptosis and cancer

Cancer cells evade necroptosis by downregulation or functional mutations of RIP1, RIP3, and MLKL genes [36]. Colon cancer cells showed decreased levels of RIP1 and RIP3 expression [37]. A hypermethylation in the promoter region of RIP3 was detected in lung cancer cells resulting in the loss of RIP3 expression [38]. Mutations in RIP1, RIP3, and MLKL were observed in human cancer tissues which modulate the RIP kinase interaction with other proteins or decrease their activity [39].

3. Polyphenols

Higher plants are found to have thousands of molecules with polyphenol structures, and edible plants are also found to contain several hundreds of these polyphenolic molecules. Plants generate these molecules as secondary metabolites that have crucial roles in plant development, reproduction, and pigmentation. They also have other roles like providing protection against diseases, parasites, ultraviolet radiation, and predators [40]. Collectively, these chemicals are known as phytochemicals which are categorized into five groups: carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Tannin, a subgroup of phenolics and to which GT belongs, is further categorized into two biologically and chemically distinct subtypes, the condensed and hydrolyzable tannins [41]. Condensed tannins, depending on their size, could be either soluble or insoluble, whereby the oligomers are soluble, and the polymers are not. On the other hand, the hydrolyzable tannins, also referred to as galloyl and hexahydroxydiphenol esters, are composed of a β-D-glucose unit, linked to at least five galloyl groups through ester bonds. GT, or tannic acid, belongs to this last group that is also characterized by the presence of digalloyl residues consisting of meta-depside bonds between two galloyl groups [42].

3.1 Polyphenols and cancer

Plant polyphenols were found to suppress tumor invasion and metastasis, such is the case of green tea polyphenols, hydrolyzable tannins, grape seed, curcumin, and resveratrol [43]. This inhibition potential is due to the downregulation of the matrix metalloproteases (MMPs) by the natural phenolics [44]. Also, epigallocatechin gallate (EGCG), the most abundant polyphenol found in tea, has been reported to decrease the vascular endothelial growth factor (VEGF), thus inhibiting tumor angiogenesis [45]. Indeed, there has been increased evidence over the years of the effect of polyphenols on the fate of cancer cells leading to growth, differentiation, and apoptosis. Phytochemicals contribute to cancer prevention by interfering in the different stages of cancer development from tumor initiation and throughout all the hallmarks of cancer [4]. Thus, one of the most studied and acclaimed biological effects of polyphenols relates to its antioxidant properties; indeed polyphenols are able to scavenge reactive oxygen species (ROS) including radical and nonradical oxygen species such as O$_2^-$, HO$^-$, NO$^-$, H$_2$O$_2$, O$_2$, and HOCl, as well as oxidatively...
generated free radicals ROC and ROOC such as those derived from biomolecules such as low-density lipoproteins (LDLs), proteins, and oligonucleic acids (DNA and RNA) [46]. Different human intervention studies on the health potential of polyphenols were conducted in healthy volunteers or on high-risk developing cancer individuals. A one-dose diet rich in polyphenols, such as fruit juices, chocolate, strawberries, and grape seed concentrate, was able to reduce the antioxidant status and protect from oxidative stress [39]. In another study, hemodialysis patients consumed 200 mL/day of red fruit juice, and the results showed a significant decrease in DNA oxidation damage and NF-κB binding activity. In summary polyphenols exert their preventive effects on cancer cells via different mechanisms, mainly via their antioxidant effects, antiproliferation and antisurvival effects, induction of cell cycle arrest, induction of apoptosis, anti-inflammatory effects, and inhibition of metastasis and angiogenesis [47].

4. Gallotannin

Gallotannin or tannic acid is a hydrolyzable tannin that is characterized by the formation of meta-depside bonds between two galloyl groups resulting in digalloyl residues [42].

4.1 Bioavailability, biodegradation, and absorption

Gallotannin is mainly present in mangoes, pomegranate, acorns, walnuts, and beverages such as wine [48]. The metabolism of tannic acid was studied in a rat model system [49]. Based on this study, the authors proposed that administered tannic acid was not hydrolyzed by the acidity of the stomach but rather by tannase enzymes produced by intestinal bacteria. Upon hydrolysis, tannic acid released glucose and small phenolic acids which include gallic acid (GA), 4-O-methylgallic acid (4-OMGA), resorcinol (RE), pyrogallol (PY), and ellagic acid (EA) [50]. It has been postulated that GT hydrolysis could be measured by the release of gallic acid. Gallic acid is further metabolized by the colon microflora into pyrogallol which is ready to be absorbed by the intestinal cells [51].

4.2 Biosafety and toxicity

Dietary polyphenols are considered as safe and well-tolerated compounds. Only few studies have reported toxicity and adverse effects induced by plant polyphenols [52]. Tested on human epithelial and fibroblast cells, PGG did not show any toxicity at concentrations below 50 μM [53]. Galla Rhois is the excrescence formed by parasitic aphids, primarily Schlechtendalia chinensis Bell, on the leaf of sumac. The gallotannin-enriched extract isolated from Galla Rhois (GEGR) was administered orally to ICR mice using three different concentrations (250, 500, and 1000 mg/kg body weight) for 14 days to evaluate its hepatotoxicity and nephrotoxicity. No toxicity on the liver and kidney organs was detected in the mice when GT was used at less than 1000 mg/kg [54]. The Chinese medicinal plant rich in PGG, Galla chinensis, was also studied for its acute and subchronic oral toxicity. No acute oral toxicity was produced in rats using the oral dose of 5760 mg/kg. The no-observed-adverse-effect level was lesser than 1500 mg/kg body weight/day in the subchronic oral toxicity study. This dose is three times higher than the recommended dose for clinical application. In addition, the Galla chinensis serum did not show any side effects to rats in the central nervous system, cardiovascular system, and respiratory
system [55]. Although these findings consider that GT and PGG are nontoxic compounds, further investigations are needed to prove their biosafety.

4.3 Functional properties

Regarding its pharmacological potential, GT has been suggested to prevent several diseases and possess different activities [56]. Gallotannin inhibited microbial growth of both Gram-positive and Gram-negative pathogens through its antimicrobial activity [57]. Gallotannin precursor, PGG, had an antiviral activity versus Herpes simplex virus type 1 (HSV-1) [58] and a high anti-inflammatory effect against atherosclerosis [59]. This effect was mediated by poly(ADP-ribose) glycohydrolase (PARG) pathway, whereby GT was found to inhibit PARG and thus trigger nuclear accumulation of poly (ADP-ribose) (PAR). Poly ADP-ribosylation is a posttranslational modification of proteins operated by poly (ADP-ribose) polymerases (PARPs). Studies have shown that inhibition of PAR formation impairs the expression of several genes involved in the inflammatory response. The antioxidant activity of PGG was associated with the inhibition of prooxidant enzymes [60]. The antidiabetic effect of PGG was mediated by the activation of insulin receptor associated with the transportation of glucose in the adipocytes and the reduction of blood glucose and insulin level in diabetic animals [61]. The anticancer effect of GT will be discussed in detail in the next section. Figure 1 summarizes the mechanisms of action of GT on cancer cells.

4.4 Mechanisms of anticancer activity of gallotannin

Gallotannin is a polyphenol that possesses interesting anticarcinogenic properties. The effect of GT in different cancer cell lines was explored by many researchers. Different programmed death mechanisms by GT were detected in different kinds of cancers and even in different cell lines of one type of cancer. Table 1 represents GT anticancer effect.

4.4.1 Gallotannin and apoptosis

Gallotannin has the ability to induce apoptosis in HCT116 (p53−/−) and HCT116 (p21−/−) colon cancer cell lines through the induction of Bax/Bcl-1

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**Figure 1.** Schematic diagram of the anticancer mechanisms of Gallotannin. GT induces apoptosis, senescence and autophagy and inhibits cellular proliferation and angiogenesis.
protein level [65]. Gold nanoparticle formulated tannic acid (AuNP-TA) was also used against colorectal cancer, and apoptosis was detected in HCT116 cell line through the upregulation of the expression of caspase3 and 9, Bak, and Bax; loss of mitochondrial membrane potential; and release of cytosolic cytochrome c [66]. In liver cancer HepG2 hepatocellular carcinoma cell line, GT attenuated the expression of pro-caspase9, pro-caspase3, Bcl2, and integrin β1 and cleaved poly(ADP-ribose) polymerase [68]. Gallotannin dramatically induced apoptosis through the expression of p53 and active caspase-3 and fragmented DNA in A549 human lung carcinoma cells [69]. In DU145, PC-3, and M2182 prostate cancer cell lines, the inhibition of Mcl-1 and activation of caspases were critically involved in GT-induced apoptosis [70]. Another study using prostate cancer cell lines showed that

| Cancer type | Cell lines | Cell death type | Molecular mechanism | Reference |
|-------------|------------|----------------|---------------------|-----------|
| Colon       | HCT116 p53−/− HCT116 p21−/− | Apoptosis | Increased Bax/Bcl2 ratio | [65] |
|             | HCT116 p53−/− HCT116 p21−/− HCT116 p53+/− | Senescence | Enhanced ROS | [64] |
| Liver       | HepG2      | Apoptosis | Pro-caspase3/9; Bcl2; PARP cleavage | [68] |
|             | HepG2      | Senescence | SA-β-gal activity; p21; IL6 | [71] [81] |
|             | SK-Hep1    | Autophagy | LC3; LC3B-II; Beclin1 | [81] |
| Lung        | A549       | Apoptosis | Caspase3; p53 | [69] |
|             | A549       | Senescence | IL6 | [81] |
|             | A549       | Autophagy | LC3-II | |
| Prostate    | DU145 PC-3, m2182 | Apoptosis | Caspases; Mcl-1 UPR pathway | [70] [72] |
|             | TRAMP-C2 PC-3 | Autophagy | AKT; S6K; 4EBP1 | [84] |
| Leukemia T-cells | Jurkat | Apoptosis | Bax | [73] |
| Breast      | MDA-MB-231 MCF-7 | Apoptosis | Caspase3; Bax; FADD | [74] [75] |
|             | MCF-7 SKBr3 | Senescence | IL6; ROS | [81] [82] |
|             | MCF-7      | Autophagy | LC3-II | [81] |
| Cervical    | HeLa       | Apoptosis | Loss of MMP | |
|             |           | Necrosis | Increase sub-G1 cells | |
| Leukemia    | HL-60RG    | Apoptosis | ROS | [77] |
| Esophageal  | TE-2       | Apoptosis | Bax; Bcl2; XIAP | [62] |
| Gingival    | GSCC       | Apoptosis | Bcl2; Bax; cytochrome c | [63] |
| Acute myeloid leukemia | HL-60 | Apoptosis | Caspases; PARP cleavage; cytochrome c | [78] |
| Glioma (brain) | HS 683 | Apoptosis | Caspase3/9; PARP; ROS | [79] |

Table 1. Anticancer effects of GT and its programmed cell death mechanisms and molecular targets.
Tannic acid can promote apoptosis via the ER stress-mediated UPR pathway [72]. The inhibition of the proteasome by TA in Jurkat T cells was associated with Kip1 accumulation of the cyclin-dependent kinase inhibitor p27 and pro-apoptotic protein Bax and was accompanied by the induction of G1 arrest and apoptosis [73]. Gallotannin regulates apoptosis via increased expression of active caspase-3 and cyclooxygenase-2 (COX-2) expression through PI3-kinase and p38 kinase pathway in MDA-MB-231 human breast cancer cells [74]. In the human breast adenocarcinoma MCF-7 cells, GT was able to cause apoptosis by increasing the percentage of apoptotic proteins Bak and FADD [75]. Gallic acid induced apoptosis and/or necrosis in cervical cancer HeLa cell line, which was accompanied by the loss of mitochondrial membrane potential (MMP; ΔΨm) [76]. Intracellular ROS induced by gallic acid, especially H2O2, plays an important role in eliciting an early signal in apoptosis in leukemia HL-60RG cells [77]. Gallic acid induced apoptosis in esophageal cancer cells (TE-2) via the upregulation of Bax and downregulation of anti-apoptosis proteins such as Bcl2 and XIAP [62]. Tannic acid induced apoptosis in gingival squamous cell carcinoma (GSCC) via the inhibition of Bcl-2 and increase of the mitochondrial localization of Bax leading to the loss of mitochondrial membrane potential, resulting in the release of cytochrome c to the cytosol [63]. The combination of tannic acid with As2O3 induced apoptosis in acute myeloid leukemia (AML) HL-60 cell line through the activation of the caspase cascade, cleavage of poly (ADP-ribose) polymerase, disruption of mitochondrial membrane potential, and release of cytochrome c [78]. Treated with GT, HS 683, a glioma cell line, showed an activation of pro-caspase 3 and caspase 9, cleavage of poly (ADP-ribose) polymerase, loss of mitochondrial membrane potential, and increased intracellular ROS production [79].

4.4.2 Gallotannin and cellular senescence

In HCT116 human colon cancer cells wildtype for p53 and p21 and null for these genes, GT caused senescence independent of p21 and p53 with the partial involvement of ROS in this senescence effect [64]. Gallotannin increased the subG1 population and induced senescence via upregulation of p21 and caused G1 arrest and higher SA-β-gal activity in hepatocellular carcinoma (HCC) HepG2 and SK-Hep1 cell lines [71]. The GT precursor (PGG) was found to induce S-phase and G1 cell cycle arrest in breast [80] and prostate cancer cells [67]. HepG2 human liver cancer cells, MCF-7 human breast cancer cells, and A549 human lung cancer underwent senescence upon treatment with PGG. These cells acquired enlarged and flattened morphology which was associated with a significant increase in the level of both IL6 mRNA and its secretory protein which is the key component of senescence-associated secretory phenotype (SASP) [81]. Senescence like S response, also known as premature senescence, is mediated by the intracellular ROS generation. PGG induced senescence like S phase arrest in HepG2, Huh-7 human hepatoma cells and SKBr3 human breast cancer [82].

4.4.3 Gallotannin and autophagy

In SK-Hep1 hepatocellular carcinoma cell line, GT induced autophagic features by increasing LC3 punctate, LC3B-II conversion, autophagic vacuoles, and decreased expression of Beclin1 [71]. HepG2 human liver cancer cells, MCF-7 human breast cancer cells, and A549 human lung cancer cells treated with PGG increased the LC3-II level, a sign of autophagy [81]. A new molecular nanoparticle
based on iron(III)-tannic complexes (Fe–TA NPs) induced autophagic cell death in HepG2 cells via upregulation of LC3 mRNA expression and autophagosome formation [83]. Autophagic responses were observed in human DU145 and PC-3 prostate cancer xenografts in nude mice detected by the formation of autophagosomes. As for molecular changes, a rapid inhibition of the phosphorylation of mammalian target of rapamycin-downstream targets S6K and 4E-BP1 was observed by PGG in PC-3 and TRAMP-C2 cells but not that of mammalian target of rapamycin itself, along with increased AKT phosphorylation [84].

4.4.4 Gallotannin and necrosis

Ammar et al. showed a necrotic effect of the crude extract of *Terminalia chebula retz.*, fruit containing tannic acid. Tested on many cancer cell lines derived from many cancer types such as breast, prostate, and osteosarcoma, the extract induced necrosis when used at higher concentrations [85]. A similar effect was detected when GT was used at higher doses in HeLa cells whereby cell death was marked by an increase of sub-G1 cells [76].

4.4.5 Antiangiogenic effect of gallotannin

Angiogenesis is defined as a process by which new blood vessels are formed and is a crucial process for tumor progression. The activation of VEGFR-2 is specific for vascular endothelial cells to promote migration during angiogenesis. Gallotannin effectively inhibited this process through downregulation of VEGF leading to the inhibition of endothelial cell angiogenesis. The inhibition of VEGF was correlated with the reduction of eNOS, a mediator of vasodilation [86]. An upregulation of SDF-1 and inhibition of CXCR4 allowed the mobilization of pro-angiogenic hematopoietic cells. Gallotannin dramatically decreased the SDF-1/CXCR4 interaction which suppressed tumor angiogenesis [87].

4.4.6 Antiproliferation effects of gallotannin

Gallotannin was found to interfere with the activation of the proliferation pathways including NF-κB, PI3K/AKT/mTOR, JAK/STAT, and many others. In colon cancer, GT inhibited NF-κB pathway [88]. In breast cancer, GT caused ROS-dependent upregulation of AMPK and downregulation of the AKT/mTOR pathway [89] and PI3K/AKT pathway [90], while it inhibited EGFR/STAT1/3 and enhanced p38/STAT1 signaling pathway [91]. Gallotannin also inhibited JAK2/STAT3 pathway in gingival squamous cell carcinoma [63]. Ras/MAPK is also suppressed by GT [87]. Gallotannin was found to inhibit JAK/STAT pathway in HCT116 colon cancer cells by our group (Gali-Muhtasib et al., unpublished findings).

5. Conclusion

With its pleiotropic molecular mechanisms of action on cells ranging from apoptosis, senescence, autophagy, necrosis, antiproliferative, and antiangiogenic effects, Gallotannin seems to be an interesting compound from natural sources with minimal side effects and high effectiveness against different types of cancer. Further research including preclinical and clinical studies may propose the use of gallotannin as an anticarcinogenic drug.
Conflict of interest

The authors declare no conflict of interest.

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