Chapter 15

Steroidal Saponins and Cell Death in Cancer

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Additional information is available at the end of the chapter

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Abstract

Steroidal saponins are natural glycosidic compounds of amphiphilic character. Their diverse biological activities are directly related to the variability of their structural constitutive frameworks, aglycones, and sugars. Several studies have demonstrated the therapeutic potential of steroidal saponins by their capacity to induce programmed cell death in different tumor cell lines. The process of cell death is required to maintain cellular and tissular homeostasis; it has been established that disturbances in the balance between cellular proliferation and cell death lead to several pathologies, including cancer. The antitumor activity of steroidal saponins has been intensely studied allowing elucidation of their different molecular mechanisms of action; this knowledge is crucial to the establishment of new therapeutic strategies against cancer.

Keywords: Steroidal saponins, cancer, cell death, apoptosis, cytotoxicity

1. Introduction

Saponins are a broad group of glycosides widely distributed in higher order terrestrial plants, and in lower marine organisms. They include a diverse group of compounds containing a steroidal or triterpenoid aglycone and one or more sugar chains [1]. Steroidal saponins are present almost exclusively in monocotyledonous angiosperms, not only in the families of *Dioscoreaceae*, *Asparagaceae*, *Liliaceae*, and *Amaryllidaceae* but also in the dicotyledonous *Solanaceae*. Triterpenoid saponins are more common in dicotyledonous angiosperms (e.g., the families *Caryophyllaceae*, *Quillajaceae*, *Sapindaceae*) [2]. Steroidal saponins are less common than triterpenoid saponins. Usually, glycosteroidal alkaloids are included in the very large alkaloid group.

Mankind has used, for thousands of years, many saponin-containing plants as soaps. Saponins have an amphiphilic character and as soaps, they are surface-active compounds and produce micelles. They have a wide spectrum of uses; in ancient folk medicine, they have been used as venoms, hemolytes, antimicrobials, and anti-inflammatories. Saponins are responsible for numerous biological effects in traditional Chinese and Japanese medicines. New uses are in the cosmetic and pharmaceutical industries, as starting materials in the semisynthesis of many high-cost products. The latter are difficult to produce through total synthesis due to their great structural complexity and numerous chiral centers. The foaming property of saponins in water resulted in the coining of the word saponin (from Latin *sapo*, soap). Properties and pharmacological activities of saponins were described in great detail in 1927, before a single saponin had been fully characterized [1].

Steroidal saponins have a wide range of pharmacological applications, including use as expectorants and to inhibit platelet aggregation, and also have hemolytic, insecticidal, anti-inflammatory, antitumor, antidiabetic, antifungal/antiyeast, antibacterial, antiparasitic, antihyperlipidemic, and anti-oxidative properties, among others [3]. Taking into account the above applications, the physiological role of saponins in animals and plants has been related to their defense systems. One of the first uses in the health field was made in the immune system, since they activate the immune response to antigens, functioning as adjuvants that improve the effectiveness of orally administered vaccines by facilitating the absorption of large molecules [4]. Later studies have allowed identifying saponins as inductors of cell death by means of several molecular mechanisms.

2. Chemical characteristics of saponins

Structurally, saponins are composed of a lipid-soluble aglycone that consists of a steroidal or triterpenoid skeleton and a water-soluble moiety, composed of sugar residues. The latter can differ in the type and amount of cyclic carbohydrates. The natural properties of saponins allow them to be dissolved in water where they form colloidal solutions that foam upon shaking [5]. The structure of saponins derived from plant sources are different from those found in animals. The same structural difference is observed in steroidal or triterpenoid saponins. In general, their water solubility depends on their sugar moiety number [6].

The triterpenoid aglycone consists of a skeleton of 30 carbon atoms, showing in general a pentacyclic structure. In triterpene saponins, ten main classes are found: dammaranes, tirucallanes, curcubitanes, lanostanes (all with a four six-membered ring skeleton), cycloartanes (possessing a cyclopropane attached to a four six-membered ring skeleton), lupanes and hopanes (in which a cyclopentane ring is attached to a four six-membered ring skeleton), oleananes, taraxasteranes, and ursanes (composed by a five six-membered ring skeleton) [7]. In all cases, several skeletons have been found to undergo ring cleavage (seco-skeletons), homologation (homo-skeletons), degradation (nor-skeletons), or rearrangements (abeo-skeletons).

All steroidal saponins contain a 27 carbon atom aglycone skeleton and are classified in three main subclasses: spirostan, furostan, and cholestane saponins [8]. Spirostan saponins contain an aglycone that is composed of four six-membered and two five-membered rings (named as A, B, C, D, E, and F-rings, Figure 1); aglycones of furostan saponins possess only A, B, C, D, and E rings (three six-membered and two five-membered rings), while aglycones of cholestane saponins have only the tetracyclic A, B, C, and D system (three six-membered and one five-membered rings). Biosynthetically, spirostans and furostans derive from a cholestane skeleton through selective oxidation pathways.

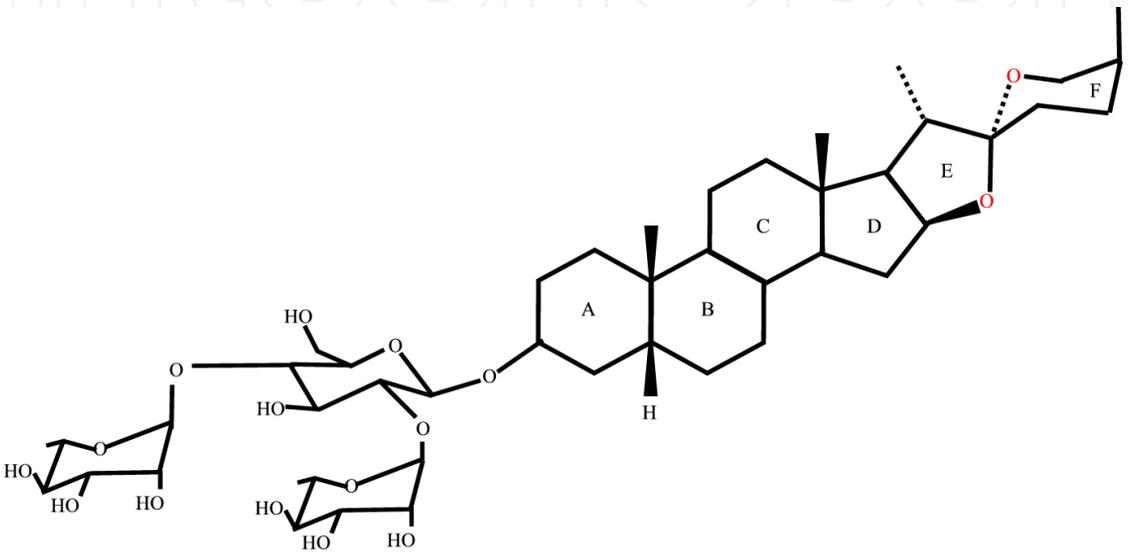

Figure 1. Structure of a spirostan saponin. Typical hexacyclic ABCDEF-ring system.

The steroidal saponin dioscin (Figure 7) had a huge importance as the favorite starting material in the steroid industry. A first transformation, an enzymatic or acidic hydrolysis, produced its aglycone diosgenin, and then modification of the diosgenin homoallylic enol and the spiroketal moieties gave progestagens, androstagens, corticosteroids, and some other important biological compounds. It is also possible to obtain a partial hydrolysis working under smooth-controlled conditions. Dioscin, and its chemically related saponins polyphyllin D and balanitins have a remarkable anticancer activity. These monodesmosidic saponins present oligosaccharide chains in which the first sugar, β-D-glucopyranose, is attached to the diosgenin C-3 position, and this in turn is substituted via its 2-OH and 4-OH positions. Commonly, α-L-rhamnopyranose, α-L-arabinofuranose, and other sugars constitute their oligosugar chains [9].

3. Diverse biological activities of saponins

As previously mentioned, steroidal saponins have an extensive variety of biological activities (Figure 2), including the absorption of cholesterol from the small intestine [10]. Mice treated with saponins from the plant *Tribulus terrestris* L. showed total cholesterol reduction in the

liver and total serum [11], and hyperlipidemia was prevented. This control of cholesterol occurs through interaction with saponins, producing insoluble complexes that are excreted in bile, thus inhibiting entero-hepatic cholesterol recycling and reducing blood cholesterol levels (reviewed in [12]).

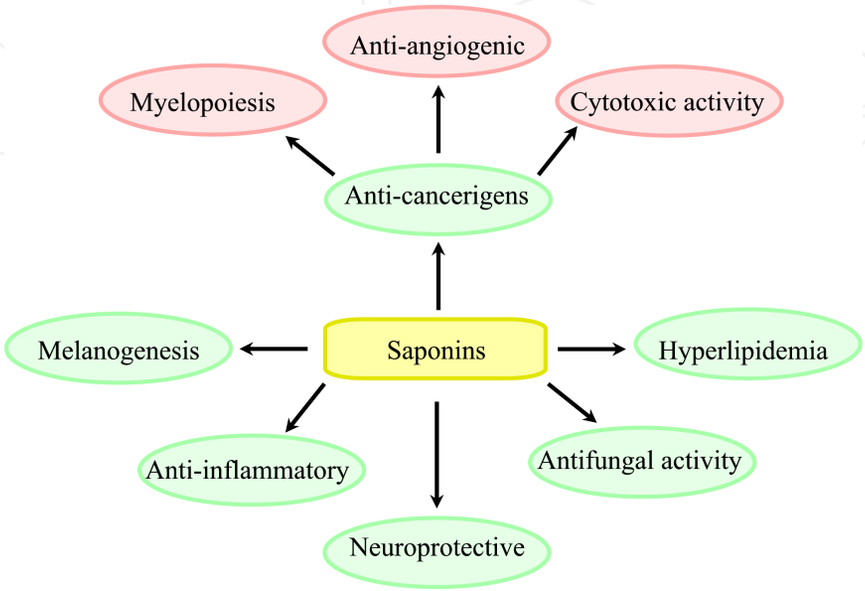

Figure 2. Biological activities of saponins.

3.1. Steroidal saponins from ginseng

Steroidal saponins are present in different types of plants. Ginseng (the root of *Panax ginseng*, C.A. Mey.) contains a series of ginsenosides that belong to the family of steroidal saponins, and these exhibit several biological properties (Figure 3). Ginsenosides are chemically structured by a skeleton consisting of four trans/anti-fusioned rings with modifications related to the type and number of sugar moieties and the attachment sites of the hydroxyl groups [13]. The two major components of the ginsenoside family are protopanaxadiol and protopanaxatriol [14]. The sugar moieties in the protopanaxadiol and protopanaxatriol are attached to the 3-position and 6-position of a dammarane-type triterpene, respectively (Figure 4). The protopanaxadiols include ginsenoside Rb1, Rb2, Rc, and Rd, while protopanaxatriols include ginsenoside Re, Rf, and Rg1.

Several reports indicate that each ginsenoside has distinct biological effects; it has been shown that purified ginsenoside protopanaxadiol Rb1 has a neuroprotective effect on PC12 (rat adrenal pheochromocytoma cell line) cells inhibiting the cell death by decreasing both the amount of active caspase-3 as well as DNA fragmentation, and increasing the amount of the anti-apoptotic Bcl-xL protein [15]. Besides acting as a neuroprotective, Rb1 has anti-angiogenic function inhibiting the process of new blood vessel formation [16], as well as an anti-inflammatory function [17].

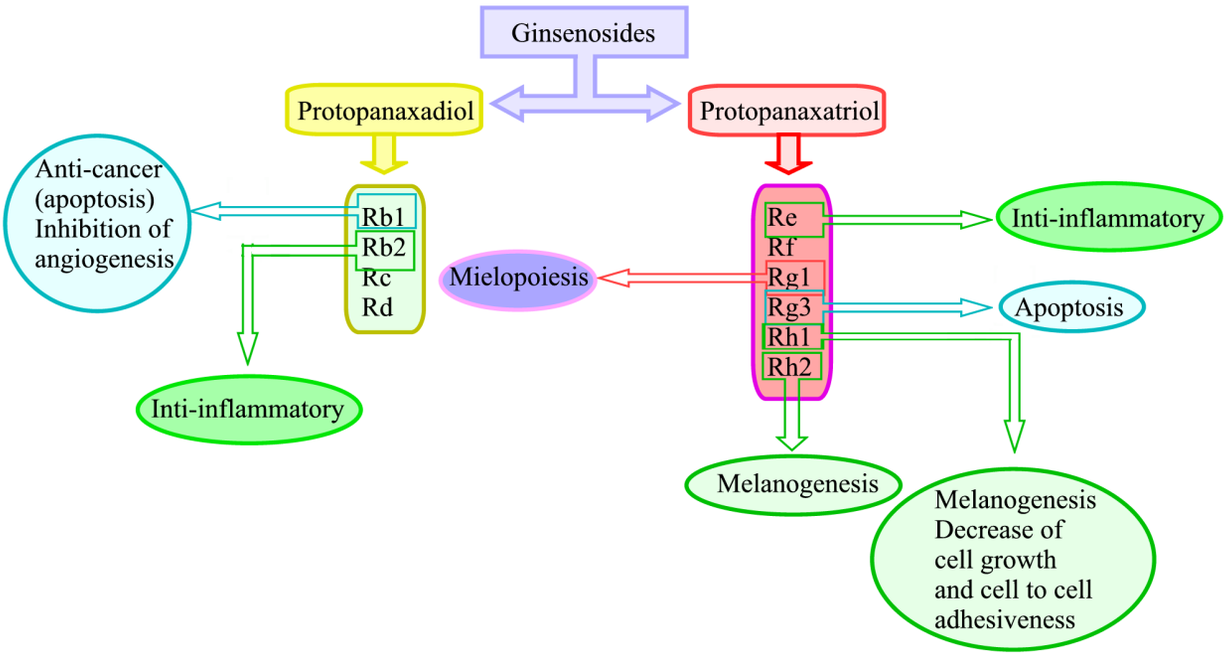

Figure 3. The two major groups of ginsenosides and their specific biological functions.

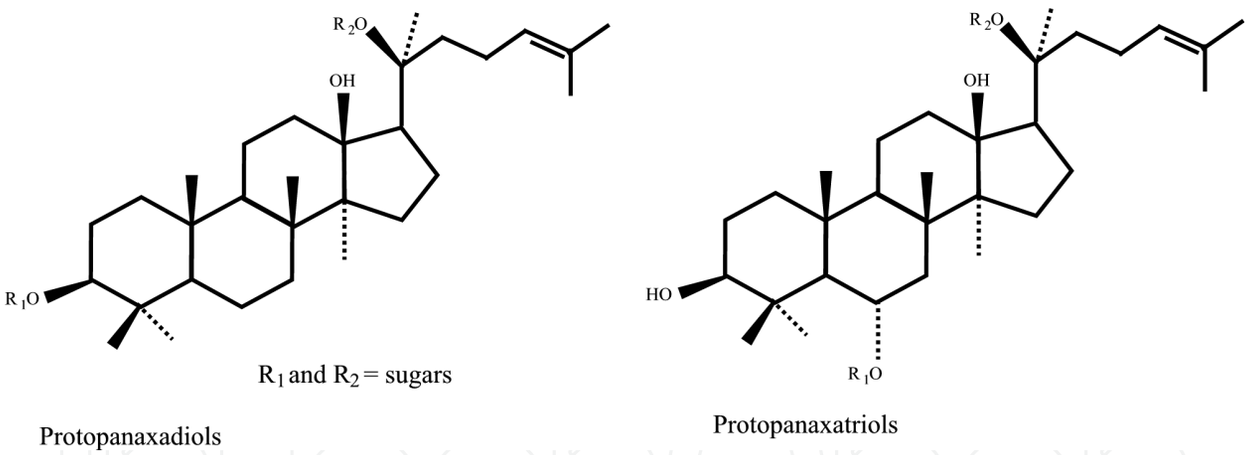

Figure 4. Structure of ginsenosides protopanaxadiols and protopanaxatriols.

The ginsenosides of the protopanaxatriol group have been shown to have several functions, some of which are similar to those exhibited by the protopanaxadiols. An anti-inflammatory effect has also has been shown by using the Re member [18]. With respect to the promotion of cell death by this group, it has been observed that Rg3 induces cell death in hepatocellular carcinoma cells in a selective form, since it does not affect normal cells [19]. The treatment of several types of tumors implies the use of chemotherapeutic agents that possess secondary reactions such as myelosupression. It has been shown that the protopanaxadiol Rg1 enhances myelopoiesis in vitro and reconstitutes bone marrow after myelosuppression treatment in mice [20].

The structural composition of saponins is important for their biological activity. Ginsenosides Rh1 and Rh2 (members of the panaxytriol group) are extracted from the root of *Panax ginseng*, but their specific activity changes according to the sugar moiety binding site. Clear examples of this are Rh1 and Rh2, which both stimulate melanogenesis, but only Rh1 decreases cell growth and cell-to-cell adhesiveness [6].

The use of botanical products containing steroidal saponins showed that the saponins present in *Panax ginseng* and *Panax quinquefolius* L. affect the central nervous system by acting on the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes (reviewed in [21]). Observations have also revealed that ginseng extract inhibits the growth of several types of tumors, such as B16 melanoma cells [22] and hepatoma cells [23].

3.2. Steroidal saponins derive from diverse plant sources

With respect to other saponins not derived from ginsenosides, several studies have demonstrated the therapeutic potential of steroidal saponins with antifungal activity. This effect has been attributed to both their individual aglycone moieties and the number and structure of their monosaccharide units. Certain pathologies associated with immunocompromised diseases as opportunistic fungal infections have been treated with the steroidal saponins C-27, which are composed of a C-27 aglycone moiety and a sugar chain with one or more monosaccharides [8]. This response could be the reason that saponins are capable of inducing a nonspecific immune response having an immunomodulatory activity by stimulating both cell-mediated and humoral immune responses [24]. Steroidal saponins denominated SC-1-SC-6 are present in *Solanum chrysotrichum* Schltdl. The SC-2 saponin is a potent antimycotic [25].

An important biological function of saponins is their capacity to induce cell death by means of programmed or nonprogrammed routes. The effect that these compounds exert inside the tumor cell has been widely evaluated, providing evidence that they could be used as agents to control cell proliferation. Steroidal saponins derived from *Withania somnifera* (L.) Dunal (Ashwagandha) have shown antiproliferative activity in rat C6 glioma cell lines [26]. Saponins can act at different cellular levels and are capable of forming pores in lipid bilayers increasing the cellular permeability, thus enabling the uptake of molecules that would otherwise be excluded and produce toxic stimuli [27]. The toxic activity shown by saponins has allowed them to be considered as possible therapeutic agents, but it is important to contemplate the possible side effects caused by their toxicity, since drugs have a dual effect; i.e., while they control certain pathologies and contribute to curing particular diseases, they can have harmful side effects that cause adverse consequences and symptoms in the body. Recent reports suggest that some saponins exhibit antiproliferative and apoptotic activity and act selectively without presenting cytotoxicity [28–32]. These findings are generating great interest in these compounds as therapeutic agents for treating cancer.

4. Antitumor activity of saponins

Cancer includes a group of complex genetic diseases that affect aged cells. Carcinogenesis is a multi-step molecular process induced by genetic and epigenetic changes that disrupt the

balance between cell proliferation, apoptosis, differentiation, senescence, and the pathways that control these cellular processes (see review in [33]).

Different types of programmed cell death are known, including apoptosis and autophagy (Figure 5). Both processes are complex and are regulated by different enzymatic activities. Apoptosis is morphologically characterized by cellular shrinkage, DNA fragmentation, and the formation of cellular fragments surrounded by a cytoplasmic membrane termed apoptotic bodies [34]. The enzymatic activity in apoptosis is developed by the caspases that are the proteases responsible for the morphological changes [35]. Autophagic cell death is characterized by an exacerbated formation of autophagic vesicles and an increased lysosomal activity [36, 37]. The hallmark of the programmed cell death processes is the absence of an immunologic response that takes place in the accidental cell death known as necrosis.

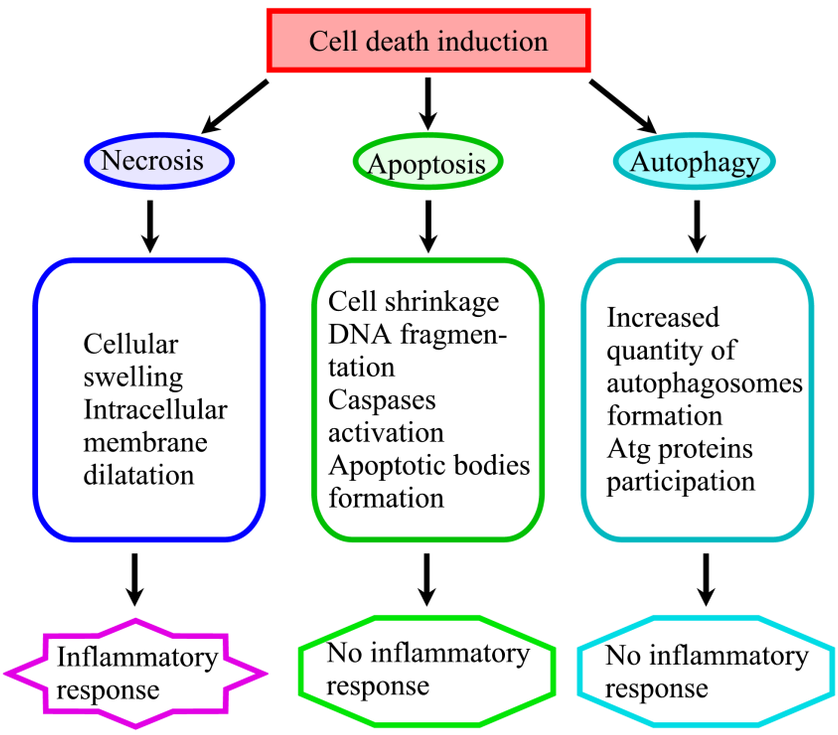

Figure 5. Characteristics of the necrosis, apoptosis, and autophagic cell death. Each cell death has its own morphological and biochemical properties.

Numerous studies using in vitro and in vivo models have been conducted to evaluate the antitumor activity of various saponins (Figure 6), including triterpene and steroidal saponins and diosgenin. Different cancer types have been treated with plant extracts that have a high quantity of steroidal saponins, as well as with isolated or synthetic steroidal saponins.

Several such assays have used plant extracts in different tumor cells. A recurrent model in the evaluation of steroidal saponins is the hepatocellular carcinoma cells HepG2. It has been shown that in the extract obtained from *Asparagus officinalis* L. whose components include steroidal saponins [38, 39], polysaccharides, and flavonoids, the steroidal saponins are the major

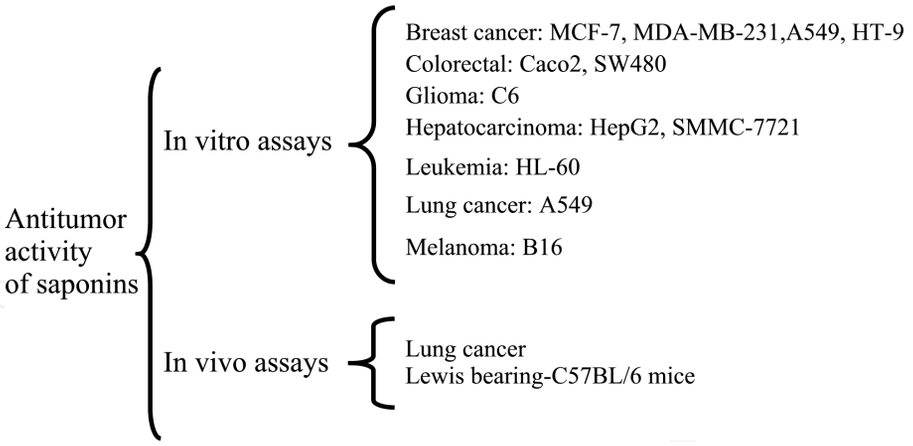

Figure 6. In vitro and in situ assays to evaluate the antitumor activity of saponins inside different types of cancer.

components responsible for antitumor activity, since in HepG2 cells they induce apoptosis [40]. Steroidal saponins isolated from *Paris polyphylla* Sm. – specifically three pennogenin steroidal saponins (monodesmosidic glucosides) – exhibit significant antiproliferation activities against HepG2 cells, inducing apoptosis [7].

Several strategies have been used to control cancer cell proliferation, including inducing cell death or cytotoxic effect on cancer cells; however, new strategies could be planned to allow more efficient chemotherapy treatments. Steroidal saponins from *Trillium tschonoskii* Maxim. reverse multidrug resistance in HepG2 and R-HepG2 cells, and significantly enhance chemosensitization to doxorubicin, thus reducing tumor formation in vivo [41].

In colon cancer cells, the anticancer effect of several plants containing saponins has been observed inside human colon cancer cell lines. *Allium flavum* L. is a plant traditionally used as a spice, but steroidal saponins from the whole plant have presented cytotoxic effects in the human colon cancer, SW480 cells, and their derived metastatic SW620 cells (a derivate of mesenteric lymph node metastasis) [42]. *Allium macrostemon* Bunge has an active steroidal saponin called macrostemonoside A (MSS.A), which suppresses cell growth in Caco2 and SW480 human colorectal cancer cell lines in two ways: by arresting the cell cycle and by inducing apoptosis [43].

Breast cancer is one of the most common malignancies in women and the second leading cause of cancer deaths [44]. It has been reported that the extract of *Dillenia suffruticosa* (Griff) Martelli shows high antioxidant and cytotoxic activities in several cancer cell breast lines, such as MCF-7, MDA-MB-231, A549, and HT-9 [45]. It has been proposed that this cytotoxic property may be due to the presence of saponins, triterpenes, tannins, and polyphenolic compounds in the extract [54]. Tor et al. (2014) [66] showed that the ethyl acetate extract exerts a series of effects in the cancer cell breast line MCF-7, including non-phase specific cell cycle arrest and apoptosis by increasing ROS (reactive oxygen species). The biological activity of *Fagonia indica* Burn. f. var. schweinfurthii *Hadidi* (family *Zygophyllaceae*) is attributed to saponin glycosides that exert a necrotic effect on the breast cancer cell lines MCF-7 and MDA-MB-468 cancer cells [46].

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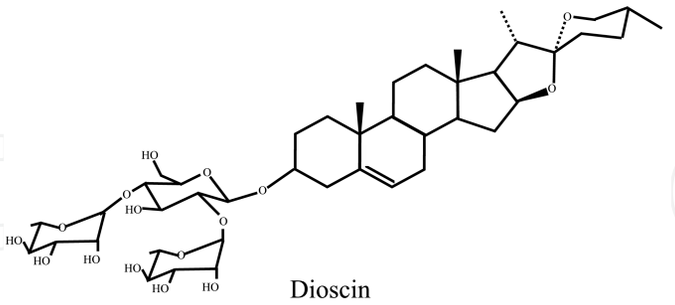
The antitumor effect of saponins has also been demonstrated in ovarian cancer cell lines. The principal constituents of *Rhizoma Paridis* (a stem of *Paris polyphylla* Sm. var. *chinensis* (Franch.) H. Hara) are steroidal saponins such as formosanin C (PSII). PSII has shown an antitumor effect in ovarian cancer cells, and studies have demonstrated that it achieved its effect by activating several mechanisms, including cell-cycle arrest and apoptosis [47].

The antitumor effect of saponins in lung cancer has been reported in both in vivo and in vitro models. The immunomodulatory role of the steroidal saponins obtained from *P. polyphylla* var. *chinensis* has been demonstrated in lung cancer using Lewis bearing-C57BL/6 mice [48]. These steroidal saponins induce apoptosis cell death in A549 lung cancer cells.

Intense research into the anticancer effects of steroidal saponins has led to the discovery of new compounds whose properties could be improved. Researchers have isolated compounds that have been used individually or in combination to induce cell death in different cancer type cells. The steroidal saponins (25 R)-5α-spirostan-3β,6β-diol 3-O-β-D-glucopyranosyl-(1-4)-[β-L-arabinopyranosyl-(1-6)]-β-D-glucopyranoside was cytotoxic for A549, HeLa, and LAC human cancer cell lines [49]. The steroidal saponin tupichinin A, together with seven known compounds isolated from rhizomes of *Tupistra chinensis* Baker, showed potent cytotoxicity against the cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 [50], while the mixed saponins balanitin-6 and -7 showed anti-tumor activity in both in vivo and in vitro systems [51]. This is evidence of the broad range and the various mechanisms of action of saponins.

Some steroidal saponins have been extensively evaluated such that each compound has an antitumor effect on different cancer types, and examples of this are dioscin and diosgenine (Figure 7). Dioscin provokes G2/M phase arrest and apoptosis in human gastric cancer SGC-7901 cells [52].



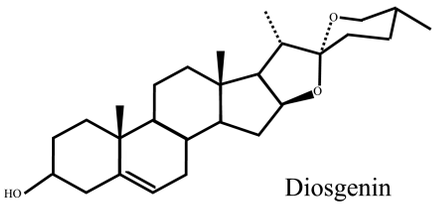


Figure 7. Structures of dioscin and diosgenin.

Diosgenin is an aglycone of steroidal saponins that exhibits antiproliferative and pro-apoptotic activities on cancer cells in vitro. Cancer metastasis involves the migration of cancer cells from the primary tumor. In this process, the matrix metalloproteinases are the main proteases that participate in tumor cell migration, spreading, tissue invasion, and metastasis [53]. Multiple studies have demonstrated the role of diosgenin as an anticarcinogenic factor, and have shown that diosgenin was able to inhibit metastasis in vitro in human prostate cancer PC-3 cells [54]. In B16 mouse melanoma cells, treatment with diosgenin inhibits melanogenesis by a mechanism of action that involves the PI3K signaling pathway [55].

Steroidal saponins have been widely used to control tumor expansion; however, it is important to take into account the characteristics of this kind of cell control, since some steroidal saponins isolated from diverse plants have not only antiproliferative characteristics but also undesirable effects. Da Silva et al. (2002) [56], for example, evaluated the anti-inflammatory activity of a steroidal saponin isolated from the leaves of *Agave attenuata* Salm-Dyck (*Asparagaceae*), but found that while it showed anti-inflammatory activity, it also has a hemolytic effect.

Steroidal saponins have been incorporated into in vivo models to control the cancer process. A recent study using nude mice bearing human hepatocellular carcinoma showed the effect of the pennogenyl saponins (PS1 and PS2) isolated from *Rhizoma Paridis*. Results from Chen et al. (2014) [57] suggest that these saponins inhibit the progression of hepatocellular carcinoma by inducing apoptosis, activating both routes of caspase activation – extrinsic and intrinsic – and inhibiting cell proliferation.

In vitro assays have provided important information on the anticancer properties of diverse steroidal saponins. However, it is important to note the need to increase the number of such experiments in order to corroborate the effects identified, while remembering that in vivo systems involve several parameters that influence the effect of the different plant extracts and the compounds isolated or synthesized from them. These include gastrointestinal absorption, kinetics, bio-availability, tissue distribution, the systemic circulation pathway, catabolism, and excretion [46].

5. Molecular cell death mechanisms of saponins in cancer cells

A desired feature in a compound used in cancer treatment is that it has the ability to remove cells in a regulated manner, with the least possible side effects. This requirement means activating the programmed cell death routes.

Previously, we mentioned that a hallmark of programed cell death is the absence of an anti-inflammatory response, which is avoided by the conservation of the cellular membrane until the late phase of the process. Apoptosis can be activated by means of two routes: the extrinsic path that involves the participation of a cytoplasmic membrane receptor, and the intrinsic route that implies the delivery of pro-apoptotic proteins by the mitochondria (Figure 8). The molecular mechanism of the apoptosis is characterized by the participation of the proteases named caspases, which can be activated by the mentioned routes. Caspases are responsible

for the morphological changes during the apoptotic process since they are able to depolymerize the cytoskeleton components.

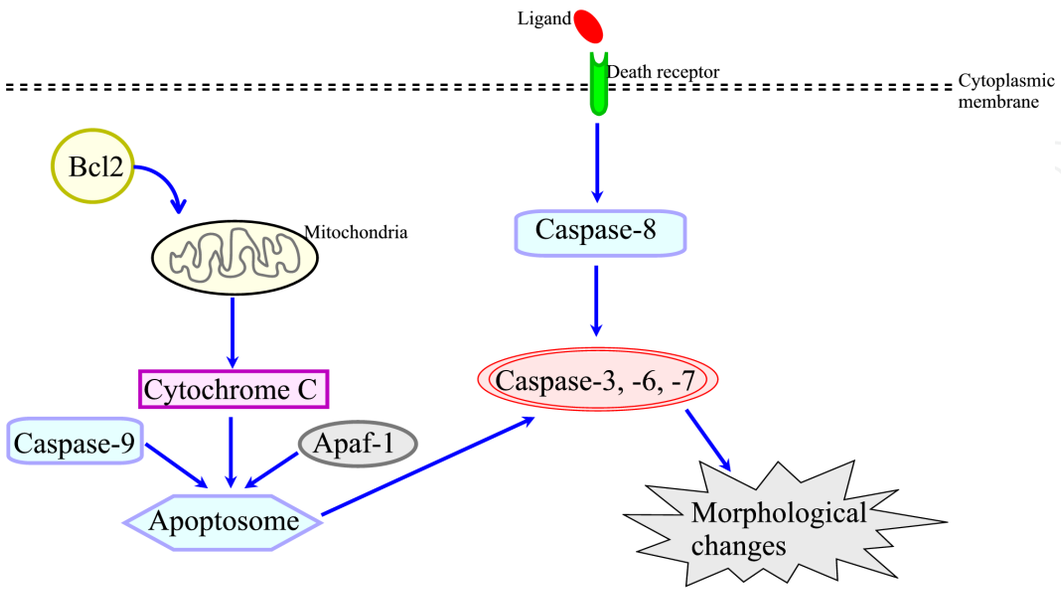


Figure 8. Intrinsic and extrinsic routes of activation of caspases. Mitochondrial activation involves the cytochrome-C delivering from the mitochondria to form a complex composed by the caspase-9 and the Apaf-1, which in turn will activate the executer caspases -3, -6, or -7. Extrinsic route implies the activation of a death receptor in the cytoplasmic membrane by means of a ligand; this process will activate the initiator caspase-8, which in turn will activate the executer caspase-3, -6, or -7. The activation of the executer caspases provokes the morphological changes related to the apoptotic process.

The molecular activity of different saponins is attributed to their structural composition. It has been demonstrated that the heterosugar moiety causes heteropolarity of steroidal saponins, leading to different membrane permeability and selectivity in the bioactivity of the compounds [7]. Saponins act at different molecular levels inside cells, and this can lead to several modifications in cellular organization.

Saponins can induce the extrinsic route of apoptosis by activating the cell death receptors present in the cell cytoplasmic membrane. As previously mentioned, the ginsenosids are used as treatment against cancer events, and several reports have identified the molecular mechanism by which they exert their apoptotic function. The 20(s)-ginsenoside Rg3 renders HCC cells are more susceptible to TRAIL (i.e., TNF-related apoptosis-inducing ligand-induced apoptosis) by upregulating DR5 (death receptor 5). An important characteristic of this system to induce cell death is that this regulation does not affect normal cells [19].

Steroidal saponins are able to promote cell death acting inside different cellular organelles, which in turn promote the release of some molecules which promote apoptosis. Some of the targets of saponins are the mitochondria and the endoplasmic reticulum; the collapse of mitochondrial potential induces a release of cytochrome-C, activating the intrinsic apoptotic pathway [58]. The endoplasmic reticulum stress triggers the release of calcium; this delivery

induces the mitochondrial apoptotic pathway [59]. It has been shown that saponins obtained from *Asparagus officinalis* induce mitochondrial and caspase-dependent apoptosis, increasing intracellular levels of reactive-oxygen-species (ROS) and calcium [40]. In the same way, the saponin dioscin induces apoptosis by the intrinsic route increasing intracellular calcium and as a consequence the mitochondria deliver pro-apoptotic proteins to active the caspases [52]. The molecule OSW-1 (3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-{*O*-[2-*O*-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1 → 3)-2-*O*-acetyl-α-L-arabinopyranoside}, isolated from bulbs of *Ornithogalum saundersiae* Baker [60], triggers an elevation in cytosolic and mitochondrial calcium concentrations. This increased calcium level then activates apoptotic factors via the interaction of OSW-1 with the endoplasmic reticulum ATPase and its endoplasmic reticulum chaperone GRP78, which is involved in endoplasmic reticulum stress responses [61]. The timosaponins are steroidal saponins of the coprostane type that have pro-apoptotic and protective autophagy functions in HeLa cells [62]. Endoplasmic reticulum stress is induced by several factors, including the accumulation of misfolded proteins. Elimination of the sections of the endoplasmic reticulum that accumulate defective proteins is carried out by the autophagy process. Autophagy is regulated by mTOR (Ser/Thr kinase target of rapamycin) (reviewed in [63]), such that when this kinase is inhibited autophagy is activated. Timosaponin TAIII induces cell death in tumor cells but not normal ones, by inducing apoptosis via endoplasmic reticulum stress, and can inhibit mTORC1 [64] while exerting its effect selectively. In fact, in vitro treatments with several saponins have shown effects on the endoplasmic reticulum.

The antitumoral effects of the saponin dioscin have been studied widely, leading to the suggestion that the results of dioscin-induced molecular expression may have a cell-type-specific correlation. It has been reported that dioscin has the ability to induce apoptosis by activating the intrinsic or extrinsic route of apoptosis execution. In HeLa cells, a cell line derived from a human cervical carcinoma, the dioscin activates the intrinsic route since this inhibits the anti-apoptotic protein Bcl-2, and activates the pro-apoptotic proteins caspase-9 and caspase-3 in HeLa cells [65]. The activation of caspase-8 is not present in HeLa cells treated with ioscin, indicating that the extrinsic routes of caspase activation do not participate in HeLa cells treated with ioscin. On the contrary, the same ioscin provokes the extrinsic apoptosis activation in human myeloma leukemia HL-60 cells, inducing FasL and FADD expression, caspase-8 activation, and Bid truncation [66], demonstrating the activation of apoptosis by cell death receptor. The vast majority of the saponins that perform this pro-apoptotic role exert their function by activating the intrinsic apoptosis pathway.

Apoptosis is a complex mechanism leading to cellular elimination, in which several factors are involved, including those that regulate the transcription process. NF-kappaB is a transcriptional factor that normally remains in an inactive form in the cytoplasm. But once activated, it is released from its inhibitor and translocated from the cytoplasm to the nucleus. Inside the nucleus, it binds in the promoter region of several target genes related to cell proliferation, angiogenesis, and metastasis [40]. Diosgenin [(25*R*)-5-spirosten-3β-ol] is a steroidal sapogenin that inhibits the invasion of tumor cells when induced by TNF (tumor necrosis factor). The diosgenin inhibits the osteoclastogenesis induced by RANKL (receptor activator of nuclear factor kappa-B ligand) by inhibiting NF-kappaB and NF-kappaB-regulated gene products [67].

This activity suggests that saponins act at the molecular level by inhibiting NF-kappaB, blocking the expression of proliferation genes, and inducing apoptotic death through the intrinsic pathway and the participation of pro-apoptotic genes.

Apoptotic cell death can be triggered by the activation of different routes of signaling besides the caspases cascade. One of the responsive routes of signaling involves the mitogen-activated protein kinase (MAPK) family members. MAPKs are serine/threonine kinases that under certain stimuli phosphorylate specific substrates, regulating diverse cellular responses including apoptosis. The saponins present in plant extracts can induce several biochemical effects that impact critical enzymes involved in signal transduction pathways such as ERK 1/2 (extracellular signal-regulated kinase 1/2). One MAPK family member, PSII – specifically – modifies ERK activities and increases the level of active caspases [47]. It has been shown that plant extracts containing saponins exerted a cytotoxic effect by increasing oxidative stress that, in turn, activated Akt (protein kinase B, a serine/threonine kinase) [68]. Akt is one enzyme involved in cell proliferation, apoptosis, and angiogenesis. The high oxidative stress induced by saponin extracts also exerts its effect inside the p53 protein (tumor suppressor protein) and the p38 MAPK signaling pathway [68]. These effects lead to cell elimination and provide saponins with antiproliferative properties.

A morphological change characteristic of apoptosis is cellular shrinkage, which is a consequence of the cytoskeleton depolymerization caused by the action of active executor caspases such as caspase-3. The effect of saponins inside elements of the cytoskeleton, whose major structural components are the microtubule and actin filaments, has also been demonstrated. The mixed saponins, balanitin-6 and balanitin-7, affected the stability of the actin cytoskeleton by depleting ATP, thus exercising antitumor activity [51]. Cellular ATP depletion in diverse cell types provokes the change of the polymerized form of F-actin into a monomeric G-actin [69]. Actin polymerization of the F-actin form allows the cell to perform diverse functions, such as mitosis, movement, signaling transduction, and substance transportation. This means that it enables correct cellular functioning.

The population of cancer cells can be regulated either by inducing cell death or by inhibiting their proliferation. Several steroidal saponins obtained from diverse plants have demonstrated their effect by inhibiting the cellular cycle progression. The saponin ioscin causes cell cycle arrest by inhibiting cyclin B1 and CDK1 [52]; the same effect has been observed in the steroidal saponin PSII (formosanin C), which also caused cell-cycle arrest [47]. Cyclooxygenases (COXs) are enzymes active in the conversion of arachidonic acid into prostanoids, which are involved in apoptosis, inflammation, mitogenesis, and immunomodulation [70]. Of their two isoforms, COX-1 is present in a constitutive form, while COX-2 is an inducible form [43]. It has been shown that diosgenin eliminates COX-2 by promoting cell cycle arrest in the G1 phase and inducing apoptotic cell death [70].

The impact of saponins at the molecular level involves altering the levels of energy required for adequate cell physiology, disrupting transduction pathway signaling, and triggering the cell death process (Figure 9).

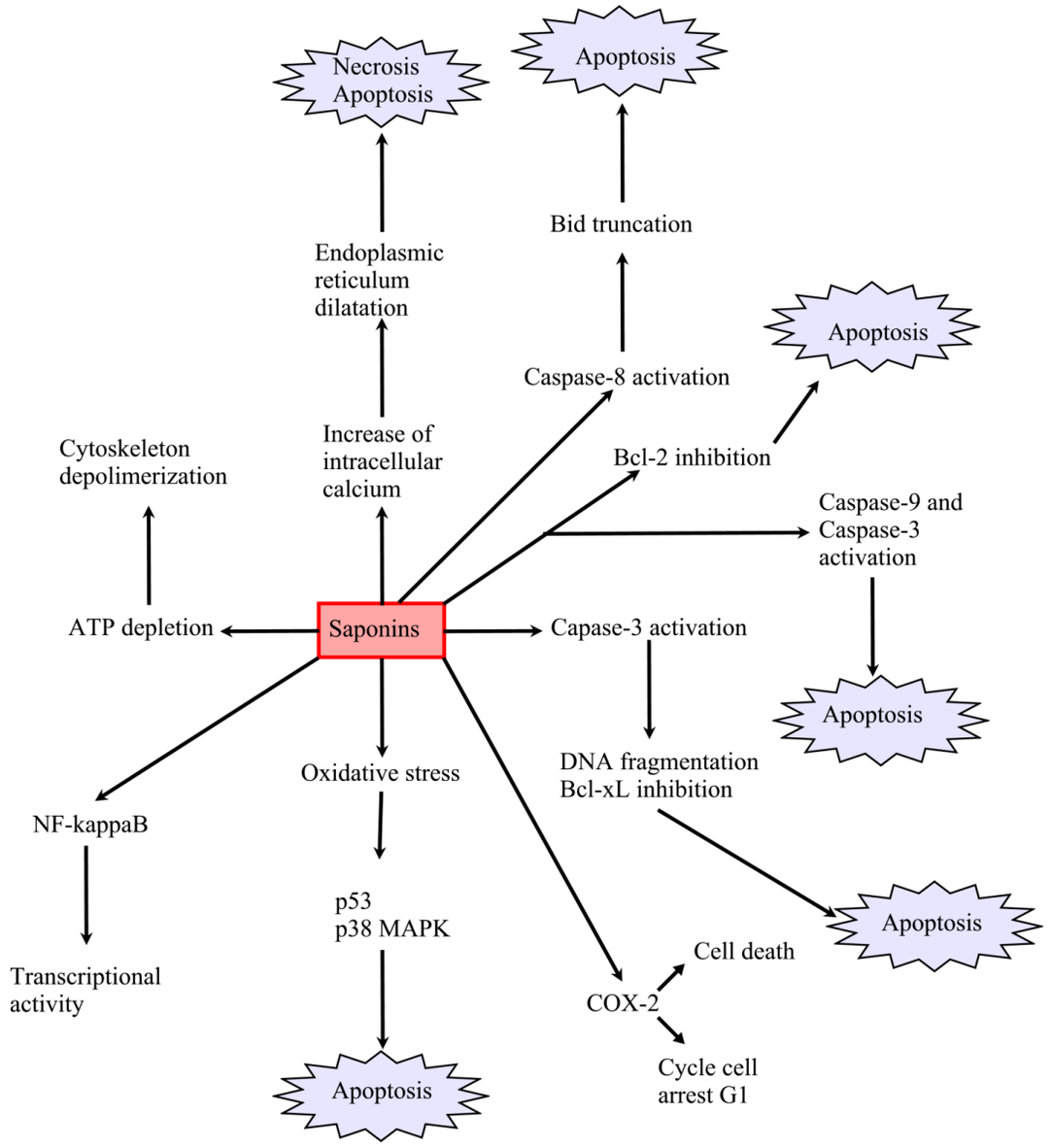


Figure 9. Different molecular cell death pathways activated by saponins. Saponins are able to induce the intrinsic and the extrinsic pathway of activation of the apoptosis cell death. In the same form, it influences inside different molecular levels including inside transcription factors as NF-kappaB as well as in the route of signaling of the MAPK. The correct polymerization of the cytoskeleton components is affected by the saponins, since this provokes a depletion of ATP inhibiting the correct polymerization of actin. In several occasions, the steroidal saponins not only induce the cell death process but also can inhibit the cell cycle progression.

6. Conclusions

Steroidal saponins are compounds that manifest antiproliferative activity and necrotic induction, and promote apoptotic or autophagic cell death in tumor cells. The important biological property of these compounds is their capacity to induce programmed cell death

(apoptosis) in different tumor cell lines. In view of the fact that the compounds used in anticancer treatments are unspecific and inefficient in terminal patients and may have side effects stemming from their cytotoxic activity, research groups are looking for new compounds with antiproliferative activity that are noncytotoxic and have selective action. This aspect is relevant because it implies that the side effects related to cytotoxic activity could be reduced quite significantly. The knowledge of different molecular mechanisms of cell death triggered by saponins is of great importance because these compounds have been shown to have significant potential as antitumor agents, and may be apt for use in treating cancers, with important cost-benefit advantages and reduced side effects.

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