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Salinomycin: A Novel Anti-Cancer Agent with Known Anti-Coccidial Activities

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Abstract

Salinomycin, traditionally used as an anti-coccidial drug, has recently been shown to possess anti-cancer and anti-cancer stem cell (CSC) effects, as well as activities to overcome multi-drug resistance based on studies using human cancer cell lines, xenograft mice, and in case reports involving cancer patients in pilot clinical trials. Therefore, salinomycin may be considered as a promising novel anti-cancer agent despite its largely unknown mechanism of action.

This review summarizes the pharmacologic effects of salinomycin and presents possible mechanisms by which salinomycin exerts its anti-tumorigenic activities. Recent advances and potential complications that might limit the utilization of salinomycin as an anti-cancer and anti-CSC agent are also presented and discussed.

Keywords

Salinomycin; cancer stem cell; toxicity; drug

INTRODUCTION

In a screen of 16,000 chemicals for efficacy to selectively eradicate cancer stem cells (CSC), Gupta *et al.* (2009) identified salinomycin as a novel anti-cancer agent on the basis that it possesses more than 100-fold higher in potency than paclitaxel, a commonly used anti-breast cancer drug [1]. Additional studies that follow this lead provide compelling evidence that salinomycin has effects on CSCs from other cancer cell types as well as activities in

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CONFLICT OF INTEREST

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overcoming chemoresistance in cancer cells. These findings offer salinomycin as a promising anti-cancer drug for chemoprevention and therapy [2, 3].

Salinomycin (molecular formula, $C_{42}H_{70}O_{11}$) is a monocarboxylic polyether antibiotic isolated from *Streptomyces albus* strain (Strain No. 80614) (Fig. 1). The usage of salinomycin in veterinary medicine can be traced back to 1980s [4] as a broad spectrum antimicrobial agent with activity against gram-positive bacteria, fungi, and parasites [4–6]. Today, salinomycin is one of the most widely used coccidiostats in poultry in the United States [6–10].

As an antimicrobial drug, salinomycin primarily functions as an ionophore that facilitate the transport of cations (K^+, Na^+, Ca^{2+} or Mg^{2+}) through cell membranes of the target organisms including protozoa and gram-positive bacteria. Most notably, such facilitated transport increases intracellular calcium to levels toxic to coccidians, by inducing the selective disposition of osmoregulatory organelles in the cell thereby disrupting the osmotic balance and resulting in eventual demise of the responsive organisms [11, 12]. However, whether such ionophoric properties and mechanisms are applicable or suffice for explaining the observed specificity of salinomycin on CSCs and multidrug resistant cancer cells remains unclear. Indeed, several studies have shown that salinomycin activates unconventional pathways of cell death, increases DNA damage, and inhibits Wnt signaling pathway, all of which purportedly have been linked to anti-cancer activities of salinomycin in various types of cancers [2, 3, 13–16]. In this review, the status and recent progress on the use of salinomycin in human cancer will be summarized and discussed.

THE DISCOVERY OF SALINOMYCIN AS A CSC ERADICATOR

Accumulating evidence shows that the presence of CSC could be the major cause of cancer recurrence after therapy. This is largely attributed to CSCs' self-renewal and tumor initiating capability which can repopulate the tumor mass and consequently confer resistance to therapy [17–20]. Clinically, CSCs also present significant challenge owing to their unique endowment with an enhanced DNA repair system, up-regulation of drug efflux pumps and robust expression of anti-apoptotic proteins [21–24]. Therefore, it is envisaged that eradication of CSCs is a key to the prevention or suppression of cancer relapse and chemo-resistance, the major obstacles in current cancer therapy. Although the epithelialmesenchymal transition (EMT) has long been recognized as a key feature of cancer invasion and metastasis [25–27], Mani *et al.* (2008) recently showed that the induction of EMT in both human mammary epithelial cells (HMLEs) and mammary carcinomas occurred in parallel with the enrichment of cells with epithelial stem cell properties [28]. Gupta *et al.* observed that EMT transformation of HMLER breast cancer cells (human mammary epithelial cells overexpressing hTERT, SV40 T/t and H-RasV12) is accompanied by the appearance of tumorigenic and chemo-resistant CSC like cells ($HMLER^{shEcad}$). Using this feature as an *in vitro* high-throughput strategy and model system, Gupta *et al.* (2009) screened over 16,000 compounds and identified that salinomycin was the only one chemical showing both selectivity and biopotency in depleting breast CSCs. Indeed, the pharmacological efficacy of salinomycin is more than 100-fold greater than that of paclitaxel, a commonly used anti-breast cancer drug [1], displaying profound inhibitory

activity on tumor seeding, growth and metastasis in NOD/SCID mice *in vivo* [1]. The extraordinary properties and presumed clinical implications of salinomycin evident in this seminal finding laid the foundation for a flurry of studies conducted thereafter examining salinomycin's anti-cancer effects in various cancer types and model systems. Table 1 summarizes the effects of salinomycin on various types of cancer and CSCs, providing compelling cumulative evidence for its consideration as a promising drug for cancer therapy [2, 3].

PHARMACOLOGICAL EFFECTS OF SALINOMYCIN ON CANCER

Following the discovery of salinomycin as a CSC killer, the pharmacological effects of salinomycin have been tested in several cell line models *in vitro* and *in vivo*. For instance, $CD133^+$ as a marker of CSC in many cancer types was utilized by Dong *et al.* to demonstrate that $CD133^+$ colorectal CSC like cells were sensitive to salinomycin treatment, but not to conventional anti-cancer drug oxaliplatin, with respect to cell proliferation, colony formation, cell migration, and invasion. The observed effects were accompanied by an upregulation of the epithelial cell marker E-cadherin expression and a suppression of the mesenchymal cell marker vimentin, thereby further implicating the inhibitory effect on the EMT process by salinomycin [29]. Consistent with this finding, Basu *et al.* observed that the mesenchymal-like subpopulations within squamous cell carcinomas show resistance to conventional cytotoxic therapy but not to salinomycin *in vitro* and *in vivo* [30]. $Kit^{low}CD44^+CD34^-$ cells in gastrointestinal stromal tumors (GIST) are clonogenic cells with the capability of self-renew and differentiation. Bardsley *et al.* showed that salinomycin blocked the proliferation of $Kit^{low}CD44^+$ $CD34^-$ cells and increased their sensitivity to imatinib in mice [31].

Human leukemia stem cell-like KG-1a cells are known to exhibit resistance to chemotherapeutic drugs via the expression of functional ABC transporters such as P-glycoprotein, breast cancer resistance protein (BCRP), and MRP8, which are capable of increasing efflux of drugs. Fuchs *et al.* observed severe cytotoxic effects of salinomycin on KG-1a cells. Unlike the conventional chemotherapeutic drugs, such as etoposide and doxorubincin, salinomycin is able to overcome ABC transporter-mediated multidrug and apoptosis resistance [32]. Riccioni and colleagues further reported salinomycin as a P-glycoprotein inhibitor by showing its inhibition of the cell growth of P-glycoprotein overexpression multiple drug resistance (MDR) cancer cell lines and the Pglycoprotein mediated drug efflux [33].

Moreover, the selective cytotoxic effect of salinomycin on tumor stem cells was also detected in osteosarcoma *in vitro* and *in vivo*. Salinomycin also sensitizes these CSCs to conventional chemotherapeutic drugs including methotrexate, adriamycin, and cisplatin [34]. Very recently, salinomycin shows profound cytotoxicity on high aldehyde dehydrogenase (ALDH) expressing stem like gastric cancer cell lines with activities surpassing 5-fluorouracil (5-FU) and cisplatin (CDDP) [35]. The combinatorial efficacy of trastuzumab and salinomycin in targeting HER2-positive cancer cells and CSCs was supported by enhanced cell death as assayed by formation of mammospheres [36]. Consistent with its promising anti-cancer activity, a few clinical case reports have

documented effectiveness of salinomycin in therapy-resistant cancer patients, e.g., a patient with metastatic invasive ductal breast cancer treated with salinomycin showed induction of clinical tumor regression [37]. Together, the aforementioned studies strongly suggest that salinomycin is a new promising agent for cancer therapy.

POSSIBLE MECHANISMS OF SALINOMYCIN ACTION ON CANCER CELL AND CSCS

Recent years, an expanding body of evidence has suggested ion channels and transporters, which exhibit important functions in cancer cell proliferation, apoptosis, invasion, and differentiation, to be an emerging target for cancer therapy [38, 39]. As mentioned previously, salinomycin belongs to the polyether antibiotic family which functions to facilitate bidirectional ion flux through the lipid barrier of membrane and thus interrupt the innate ion transport systems in both prokaryotic and eukaryotic cells. Being a highly selective potassium ionophore, salinomycin may interfere with potassium channels and promotes the efflux of potassium ions from mitochondria and cytoplasm. [9] However, whether or not salinomycin's ionophoric activity offers an adequate therapeutic index demands further investigation.

On the other hand, activation of unconventional pathways of cell death, enhanced DNA damage, and inhibition of Wnt signaling pathway, appear to be plausible mechanisms for the multi-dimensional anti-CSC and anti-tumorigenic activities of salinomycin [2, 3, 13–16]. Fuchs and colleagues showed that salinomycin induces apoptosis in human cancer cells and overcomes apoptosis resistance through a pathway independent of activation of p53, caspase, CD95/CD95L system, and the proteasome [13]. Kim *et al.* observed that salinomycin induces massive apoptosis accompanied by caspase-3 activation and cleavage of PARP-1 in human prostate cancer cells and proposed that these effects may be attributed to accumulated ROS and mitochondrial membrane depolarization [40]. Ketola and colleagues showed that increased levels of oxidative stress play important roles in salinomycin induced prostate cancer cell growth inhibition [41]. Although many pathways have been proposed, none of these pathways could fully explain the specificity of salinomycin to CSCs (Fig. 2).

Kim *et al.* demonstrated that combined administration of salinomycin with doxorubicin or etoposide led to increased DNA damage and resulted in massive apoptosis in drug resistant cancer cells [14]. Mechanistic studies revealed that by increasing DNA damage through the enhanced expression of p53 and H2AX and reducing the expression of anti-apoptotic p21, salinomycin sensitizes cancer cells to DNA damaging agents, such as doxorubicin and etoposide [14]. In a separate study, salinomycin treatment increased DNA damage and induced G2 arrest, thereby, sensitizing cancer cells to radiation treatment. Similarly, salinomycin could suppress the elevated p21 level resulted from radiation treatment and promote the expression and activation of H2AX and p53 [15]. Very recently, Dhaheri and coworkers observed an increase in histone H3 and H4 hyperacetylation by salinomycin treatment in MDA-MB-231 breast cancer cells [42]. These studies highlighted the roles of DNA damage and histone modifications in salinomycin's action and suggested that patients

may benefit from the combined use of salinomycin with other chemotherapeutic drugs and radiation therapy.

A recent report from Verdoodt and colleagues raised, for the first time, the aspect of autophagic cell death as another mechanism of cell death elicited by salinomycin in colon and breast cancer cells [43]. This conclusion was based on the phenomenon that cell death induced by salinomycin occurred with accompanying features of autophagy, e.g., formation of multiple vacuoles and increased uptake of autophagy markers. Moreover, the induction of ROS and its consequent activation of JNK pathway have also been observed and linked to salinomycin induced autophagic cell death [43]. In addition to the inhibition of cell viability and induction of cell death, Kuo *et al.* observed that salinomycin induced differentiation of head and neck squamous cell carcinoma (HNSCC) stem cells concomitant with the activation of EMT and the phosphorylation of Akt [44].

Wnt signaling is critical for mammalian development, stem cell renewal, and cancer progression [45, 46]. Lu *et al.* have discovered that nanomolar concentrations of salinomycin exhibit profound inhibition on Wnt signaling which is constitutively activated in chronic lymphocytic leukemia cells [16]. This study further showed that salinomycin disrupts the Wnt signaling by impeding the phosphorylation of lipoprotein receptor related protein 6 (LRP6), a Wnt coreceptor, and inducing its degradation [16]. Recently, He and colleagues reported the suppression of the expression of Wnt/β-catenin signaling related proteins, such as β-catenin and p-GSK-3β by salinomycin in pancreatic cancer cells [47]. Nuclear factor-κB (NF-κB) is critical for multiple cellular functions, such as cell proliferation and defense of oxidant induced cellular damage, which are key elements for tumorigenesis and metastasis [48, 49]. Moreover, NF-κB pathway is activated in prostate stem-like tumor-initiating cells and the suppression of NF-κB results in prostate CSC apoptosis [50]. By using a luciferase reporter assay, Ketola *et al.* showed that growth inhibition and induction of oxidative stress in prostate cancer cells resulted from treatment by salinomycin was mediated via the suppression of NF-κB pathway activity [41]. More recently, Zhang and colleagues discovered that salinomycin-induced apoptosis in OV2008 ovarian cancer cells was associated with activating p38 MAPK signaling, a critical pathway in cancer development [51, 52]. However, the exact mechanism of how salinomycin regulates the complex cell signaling in cancer and CSCs remains largely unknown underscoring the requirement for further studies and elucidation.

PHARMACOKINETIC PARAMETERS OF SALINOMYCIN AS AN ANTI-COCCIDIAL CHEMICAL

The pharmacokinetic parameters of salinomycin as an antimicrobial and anticoccidial antibiotic have been extensively studied in many animal species. By virtue of its lipid solubility, it is readily and rapidly absorbed in the gastro-intestinal tract and distribute throughout the serum and tissues. In respect to tissue distribution, fat tissues showed the highest affinity for salinomycin followed by liver and muscle tissues in chicken [53]. Salinomycin is able to penetrate blood brain barrier, though the existence of P-glycoprotein could limit its oral availability and brain penetration [54]. Liver is the primary site for metabolizing salinomycin; rapid hepatic biotransformation of salinomycin yields numerous

metabolites [55]. The elimination of salinomycin is moderately fast; 24 hours has been suggested as an adequate withdrawal period for salinomycin in chickens [53]. The oral LD50 values of salinomycin in broiler chickens and laying hens were 108 and 104 mg/kg body weight respectively [56], while the reported LD50 value of salinomycin in horse is only 0.6 μg/kg [57, 58]. Although the pharmacokinetic parameters of salinomycin as an anticoccidial agent have been established and may provide insights on pharmacokinetic/pharmacodynamic studies of salinomycin in CSCs and human cancer cells, the latter aspects are far from clear and in need of further investigation. It is noteworthy that in a recent reported case, a patient with advanced and metastatic squamous cell carcinoma of the vulva received intravenous administration of 200–250 μg/kg salinomycin every second day in combination with chemotherapeutic drug erlotinib, favorable clinical effects were observed providing a pharmacodynamic window for future studies in humans [37].

CHALLENGES IN USING SALINOMYCIN AS AN ANTI-CANCER AGENT FOR HUMANS

The foregoing presentation and discussion on the significant anti-cancer effects of salinomycin must be considered in the context of its relatively narrow therapeutic index and potential toxicities. It is noteworthy that salinomycin has relatively few side effects in normal cells. Lu *et al.* showed that salinomycin has negligible effect in inducing apoptosis in peripheral blood mononuclear cells (PBMCs) at concentrations 100-fold higher than that in malignant cells [16]. Scherzed and colleagues also found that only when salinomycin at doses > 30 μM exhibited negative effects on cell viability, migration capability and the ability to form spheroids by human bone marrow-derived mesenchymal stem cells (hBMSC) [59]. However, additional studies using other “normal” cells, especially bone marrow cells, are required before definitive conclusions can be drawn. Recently, Boehmerie and Endres evaluated the cytotoxicity of salinomycin on human neuronal cells and showed that salinomycin treatment led to increased cytosolic Na^+ concentration, which consequently resulted in elevated cytosolic Ca^{2+} and activated calpain, as well as induced the release of cytochrome c from depolarized mitochondria and caspase-dependent apoptosis [60]. These results suggest that it may be prudent to develop efficient strategies, such as tissue specific drug delivery, to prevent the associated neurotoxic side effects of salinomycin. In fact, overdose or accidental ingestion of salinomycin has shown severe toxic effects in cats, dogs, pigs, horses, as well as human [58, 61–64]. Moreover, as mentioned, salinomycin is known to interfere with potassium channel implying that, besides cancer cells and CSCs, it could be toxic to normal neural cells and hematopoietic stem cells. Recently, Huczynski and colleagues synthesized a set of amide and benzotriazole ester derivatives of salinomycin and demonstrated their profound anti-bacterial, antimicrobial, and anti-cancer activities [65, 66]. In consideration of its potential unfavorable cytotoxicity, future studies on the structure-activity relationship (SAR) of salinomycin will be invaluable in aiding the development of less cytotoxic and more selectively potent derivatives for clinical use.

Zhang and colleagues have made the first effort on the development of CSC targeting salinomycin by incorporating it in micelles [67]. Since nanoscale drug delivery system loaded with drug for CSCs has been shown by previous studies to be more efficient to solid

tumors than free drug [68], Zhang *et al.* synthesized salinomycin loaded PEG-b-PCL polymeric micelles (M-SAL) which exhibit distinctly superior efficiency in suppressing breast CSCs *in vivo* compared with free salinomycin. Moreover, the M-SAL was more potent in eradicating the breast CSC population than cancer cells [67]. In another effort for breast cancer target drug delivery, Aydin and colleagues synthesized a Herceptin (HER)-immobilized salinomycin (SAL)-encapsulated poly(lacticco- glycolic acid) (PLGA) (HER-SAL-PLGA) nanoparticles which were successfully taken up by MCF-7 breast cancer cells [69]. These studies shed light on the development of salinomycin with improved bio-distribution and preferential encapsulation and accumulation in tumors, while concurrently minimizing its toxic effects. Further investigations in the design and development of prominent drug delivery strategies for salinomycin in cancer and CSCs will prove invaluable for salinomycin's successful clinical deployment in the future.

CONCLUSION AND FUTURE DIRECTIONS

By directly targeting CSCs, salinomycin offers a new paradigm and promising treatment approach by restricting drug resistance and disease relapse. The considerable information that has been garnered on its efficacy suggests that pre-clinical and clinical studies of salinomycin are timely and necessary as the next step to provide direct evidence whether salinomycin can serve as a therapeutic reagent for specific cancer types. In addition, though many signaling pathways and cellular processes have been linked to salinomycin's anti-cancer activity, the precise mechanism of salinomycin's action in CSCs and chemo-resistant cancer cells remains unclear and is urgently awaiting future investigations. Development of superior delivery strategies to optimize the bio-distribution of salinomycin is also worthwhile for the potential clinical use of salinomycin.

Finally, identification of salinomycin's direct targets and indirect targets in cancer or CSC will provide critical information to understand its underlying mechanism. Such knowledge will provide new insight into cancer therapy, a theoretical base for the use of salinomycin as anti-cancer agent and allow for rapid screening for potentially therapeutic compounds with high potency and minimal side effects.

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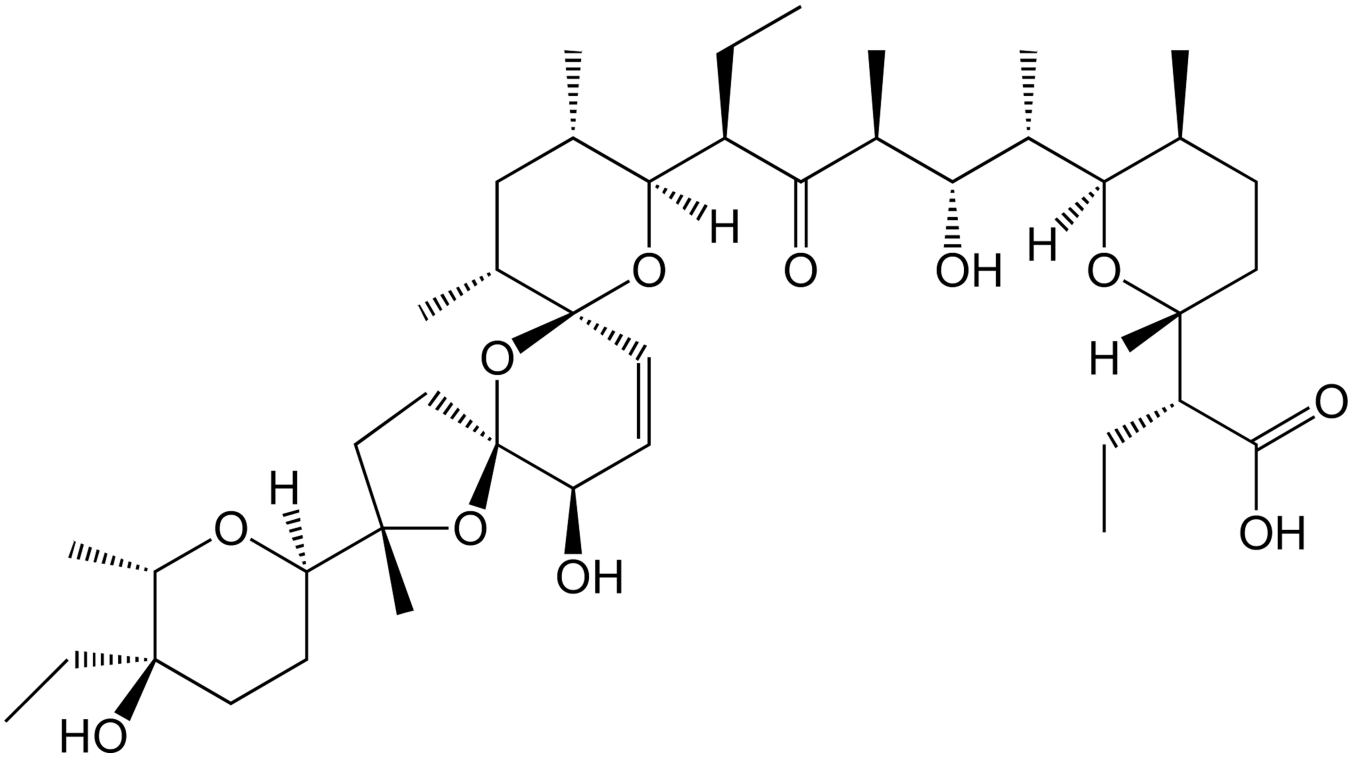

Fig. (1). Structure of salinomycin

Salinomycin is a 750 Da monocarboxylic polyether antibiotic with unique tricyclic spiroketal ring systems and an unsaturated six-membered ring. The molecular formula of salinomycin is $C_{42}H_{70}O_{11}$.

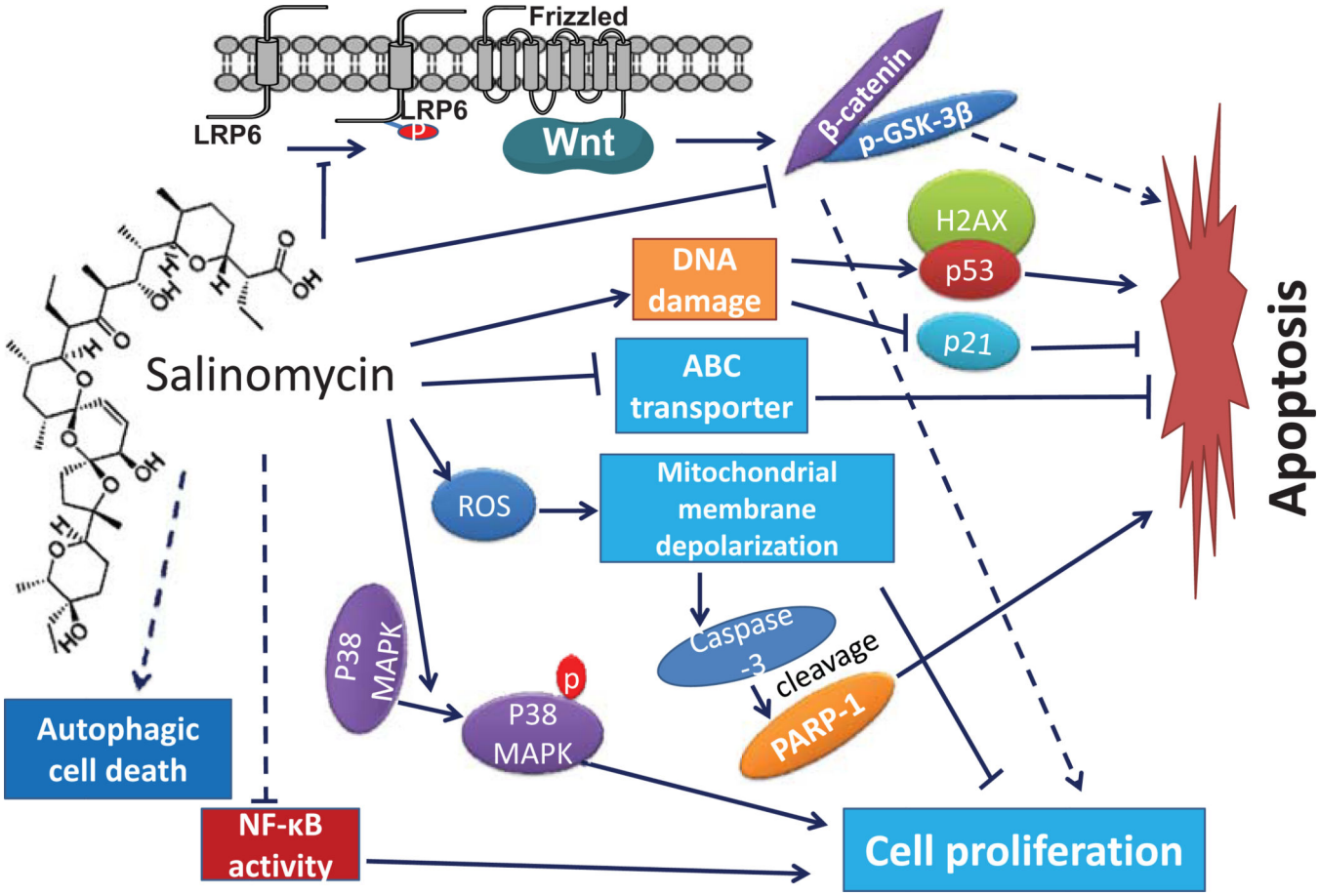

Fig. (2). Anticancer mechanisms of salinomycin

Salinomycin exerts its inhibition on Wnt signaling pathway through its suppression on the phosphorylation of LRP6 and the expression of β-catenin and p-GSK-3β. It also induces the production of ROS and mitochondrial membrane depolarization, which consequently results in the activation of caspase-3, the induction of PARP-1 cleavage, and the elicitation of DNA damage. These events subsequently induce tumor cell death and inhibit cancer cell growth. Moreover, salinomycin is able to induce autophagic cell death, inhibit NF-κB pathway, and activate p38 MAPK pathway through so far unclear mechanisms [14–16, 40, 41, 43, 47, 50, 51].

Table 1

Investigations of the Anti-Cancer Effects of Salinomycin in Human Cancers

Cancer Type	Cancer Cell	Cancer Stem Cell or Cancer Stem Like Cell	Chemo- or Radio-resis-tant Cancer Cell	Reference
Breast Cancer	√	√	√	[1, 2, 14, 36, 42, 43, 67, 69, 70]
T Cell Leukemia	√		√	[13]
Human Acute Lymphoblastic Leukemia	√		√	[13]
Human Uterus Sarcoma	√		√	[13, 14]
Burkitt's Lymphoma	√		√	[13]
Human Promyeloblastic Leukemia	√	√	√	[32]
Murine Gastrointestinal Stromal Tumors		√		[31]
Human Liver Hepatocellular Carcinoma	√			[14]
Human Lung Adenocarcinoma	√	√		[71]
Human Colorectal Cancer	√	√		[29, 43]
Squamous Cell Carcinomas	√		√	[30, 44]
Chronic Lymphocytic Leukemia	√			[16]
Osteosarcoma		√	√	[34]
Prostate Cancer	√			[40, 41, 50]
Gastric Cancer		√		[31, 35]
Pancreatic Cancer	√			[72]
Human Ovarian Epithelial Cancer	√			[33]
Human Ovarian Cancer	√			[51]
Human Cholangiocarcinoma	√			[73]