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Review

Potential role of genipin in cancer therapy

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ABSTRACT

Genipin, an aglycone derived from the iridoid glycoside, geniposide, is isolated and characterized from the extract of *Gardenia jasminoides* Ellis fruit (family Rubiaceae). It has long been used in traditional oriental medicine for the prevention and treatment of several inflammation driven diseases, including cancer. Genipin has been shown to have hepatoprotective activity acting as a potent antioxidant and inhibitor of mitochondrial uncoupling protein 2 (UCP2), and also reported to exert significant anticancer effects. It is an excellent crosslinking agent that helps to make novel sustained or delayed release nanoparticle formulations. In this review, we present the latest developments of genipin as an anticancer agent and briefly describe its diverse mechanism(s) of action. Several lines of evidence suggest that genipin is a potent inhibitor of UCP2, which functions as a tumor promoter in a variety of cancers, attenuates generation of reactive oxygen species and the expression of matrix metalloproteinase 2, as well as induces caspase-dependent apoptosis *in vitro* and in *in vivo* models. These finding suggests that genipin can serve as both a prominent anticancer agent as well as a potent crosslinking drug that may find useful application in several novel pharmaceutical formulations.

1. Introduction

In contemporary medicine, synthetic drugs play an important role in the cure of several diseases. However, these drugs have several adverse effects that impact the growth of healthy cells and disrupt normal cellular homeostasis. Therefore, compounds isolated from natural sources are constantly being exploited as effective, non-toxic alternatives to standard-of-care chemopreventive and therapeutic drugs. These compounds can either be used alone or in combination with standard chemotherapeutic agents to minimize the toxic adverse effects that are often encountered in cancer chemotherapy. Hence, there is a focus on the identification and development of novel bioactive compounds from natural sources [1–16]. Genipin is the active constituent isolated from the fruit of *Gardenia jasminoides* Ellis, which belongs to the family Rubiaceae. Genipin is the aglycone derived from the iridoid glycoside known as geniposide. In traditional oriental medicine, the fruits are eaten as a whole and the glycoside geniposide gets converted to genipin by bacterial enzyme β-D-glycosidase in the digestive organs prior to entering the blood circulation [17,18]. It is widely used in traditional medicine as an anti-inflammatory [19–23], antiangiogenic [24], antioxidant [25–27], antidiabetic agent [28–30], and for the treatment of a variety of inflammation-associated diseases, including cancer [31–33]. In addition, genipin was found to exhibit significant hepatoprotective activity [34–36]. In this review, we comprehensively discuss the *in vitro* and *in vivo* anticancer properties of genipin and its diverse molecular targets and also highlight upon its role in modulation of various established hallmarks of cancer.

2. *In vitro* anti-cancer effects of genipin

Genipin has been reported to inhibit the proliferation of several cancer cells, including leukemia, breast, prostate, and hepatocellular carcinoma [20,37–40]. Cancer cells are inherently and genetically dependent on aerobic glycolysis, which is defined as aerobic breakdown

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of glucose into adenosine triphosphate (ATP) [41]. It was Otto Warburg who noticed that cancer cells had an irreversible injury to respiration, which is characterized by low oxidative phosphorylation and high aerobic glycolysis [42–44]. In addition, mitochondrial dysfunction also plays a prominent role in the transformation of cancer cells to a malignant phenotype [45–47]. Recent studies have implicated several mutations or deletions in mitochondria DNA leading to irreversible injury to respiration [48]. Genipin inhibited the growth of cisplatin- and tamoxifen-sensitive MCF-7 and T47D breast cancer cells by inhibiting uncoupling protein 2 (UCP2), and induced apoptosis and autophagy. The authors concluded that UCP2 could be a therapeutic target in breast cancer patients treated with tamoxifen [49]. Several lines of evidence(s) suggest that overexpression of UCP2 is frequently observed in leukemia, colon, and hepatocellular carcinoma cells [43,46,47].

Numerous studies have shown that the inhibition of UCP2 enhances the action of several anticancer agents [43]. UCP2 has been implicated in the survival of several cancer cells, including breast cancer cells [50]. Genipin inhibited the ectopic expression of UCP2 in MCF-7 breast cancer cells by inhibiting cancer cell growth, invasion, and migration *in vitro* [51]. Genipin was shown to dose-dependently inhibit androgen-independent PC-3 prostate cell proliferation by suppressing UCP2, intracellular pyruvic acid, and mitochondrial succinate dehydrogenase [52]. In another study, genipin modulated glucose metabolism and mitochondrial function in T47D breast cancer cells [53]. In this study, genipin inhibited positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro- D-glucose (^{18}F-FDG PET) uptake and was associated with a reduction in both glycolytic flux and mitochondrial oxidative respiration [53].

Interestingly, Pozza et al. [54], showed that UCP2 inhibition by either genipin or by UCP2-specific siRNA synergistically reversed gemcitabine resistance, and enhanced the activity of gemcitabine and induced apoptosis of cancer cells. In this study, the role of UCP2 was first time linked to the development of cancer cell resistance to gemcitabine; therefore, UCP2 can be a potential biomarker for cancer resistance therapy [54]. In pancreatic adenocarcinoma cells, genipin or UCP2 siRNA inhibited cell proliferation and induced the nuclear translocation of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and increased the expression of the autophagic marker, LC3-II and autophagosomes. In addition, genipin potentiated the cytotoxic activity of gemcitabine and induced autophagic cell death [55].

In another report, genipin sensitized multidrug resistant cancer cells to chemotherapeutic agents, such as doxorubicin and epirubicin [56]. In breast cancer cells, genipin induced apoptosis and inhibited invasion and migration of highly invasive triple-negative breast cancer MDA-MB-231 cells [31]. In non-small cell lung cancer H1299 cells, the genipin dose-dependently induced apoptosis was associated with an increase in caspase-3 and caspase-9, cytochrome *c*, and Bax/Bcl-2 ratio [32]. In AGS gastric cancer cells, genipin inhibited cell growth and induced caspase-3-dependent apoptosis. Genipin also modulated the p53-independent early growth response-1 (Egr1)/p21 signaling pathway in a dose-dependent manner [40,57]. In HepG2 and MHCC97 L metastatic hepatocellular carcinoma (HCC) cells, genipin treatment suppressed the growth and proliferation of cells, invasion, and migration [58]. Genipin was reported to abrogate the signal transducer and activator of transcription 3 (STAT3) pathway in multiple myeloma and lymphoma cells by upregulating the Src homology2 domain-containing phosphatase 1 (SHP-1), which is the endogenous inhibitor of STAT3 [59–62]. Genipin was also found to downregulate STAT3-regulated gene products, such as Bcl-2, Bcl-xL, surviving, cyclin D1, and vascular endothelial growth factor, and induced apoptosis in U266, U937, and MM.1S cells. In addition, genipin potentiated the cytotoxic effect of the standard chemotherapeutic compounds - bortezomib, thalidomide, and paclitaxel - in U266 myeloma cells [59].

Moreover, genipin either alone or in combination with cisplatin inhibited HCT-116 colon cancer growth by suppressing UCP2-mediated proton leak, promoted reactive oxygen species (ROS) formation, and sensitized cells to cisplatin [63]. In a recent study by Kim JM et al. [64] it was found that genipin at low doses induced nuclear factor-erythroid-2-related factor 2 (Nrf2) and upregulated glutathione peroxidase, and reduced the ROS levels. However, at high dose levels, genipin induced cytotoxicity in the AGS human gastric cancer cell line [64]. In another study using 2D gel electrophoresis, 19 proteins were identified as being differentially expressed in pancreatic cancer cells lines, Panc1 and PaCa44, after treatment with genipin. Interestingly, UCP2 sensitizes pancreatic cancer cells to the glycolytic inhibitor 2-deoxy-D-glucose, and in cells transfected with siUCP2, cell growth inhibition by genipin was reversed [65]. In Hep3B HCC cells, genipin induced apoptosis by activation of NADPH oxidase-ROS-cJUN NH2-terminal kinase (JNK)-dependent activation of the mitochondrial apoptotic pathway [66].

In another study, Hong and Kim [67], reported that genipin stimulated mixed lineage kinase 3 (MLK3) expression in a dose- and time-dependent manner in PC3 prostate cancer cells. In addition, genipin-induced apoptosis was mediated by ROS dependent MLK3 activation [67]. In human cervical carcinoma HeLa cells, genipin dose-dependently inhibited cell proliferation, and induced apoptosis. Apoptosis was confirmed by an increase in DNA fragmentation and sub-G_1 cell population, and an increase in p53 and Bax protein levels after treatment with genipin [37]. Genipin, at doses of 200–400 μM/L, inhibited K562 leukemia cell proliferation, which was associated with the activation of caspase-3 and G2/M phase cell cycle arrest [68].

Genipin was also isolated and identified in the methanolic extract of *Apodytes dimidiate*, which belongs to the family Icacinaceae. The methanolic fraction containing genipin inhibited the growth of Ehrlich's ascites carcinoma, Jurkat human T lymphocyte cells, and SKBR3 mammary tumor cells in a dose-dependent manner [69]. Genipin that was isolated from the bark and fruit of Rothmannia wittii was found to be active against the NCI-H187 lung cancer cell line [70]. UCP2 expression in human uterine cervical cancer cells was inhibited by genipin, and it significantly increased cisplatin sensitivity when UCP2 was inhibited *in vitro*. Thus, UCP2 expression may become a predictive marker of whether neoadjuvant chemotherapy is effective for patients with locally advanced uterine cervical cancer, which could potentially improve patient prognosis [71]. The key molecular mechanism(s) of action of genipin are summarized in Fig. 1 and the various molecular targets modulated by this aglycone are indicated in Table 1.

3. *In vivo* anti-cancer effects of genipin

In an orthotopic HCC-implantation mouse model, oral administration of genipin at 30 mg/kg every 2 days for 4 weeks significantly reduced tumor size at the end of treatment, without pathological changes to the gastrointestinal tract, kidney, and lung, indicating minimal toxicity [72]. In addition, the number of CD31- and Ki67-positive cells within HCC was found to be reduced significantly, suggesting suppressive effects of genipin on blood vessel formation and proliferation of cancer cells. In addition, infiltration of tumor-associated macrophages (TAMs) was also found to be suppressed upon genipin treatment, which was attributed to its role in inactivation of inositol-requiring 1alpha (IRE1α) proteins present on TAMs. As IRE1α induces x-box binding protein (XBP) silencing and activation of nuclear factor kappa B (NF-κB) machinery, treatment by genipin potentially reduces inflammation in the HCC microenvironment. Therefore, genipin may represent a promising immunomodulatory therapeutic candidate for HCC [72]. UCP2 over-expression also promotes growth of orthotopic tumors *in vivo* in athymic nude mice. MCF-7 parental or MCF-7-UCP2 over-expressing cells were injected orthotopically into the mammary fat pad of female nude mice two days after subcutaneous implantation of 17β-estradiol pellets. Tumor volume was significantly higher in MCF-7-UCP2 over-expressing cells compared of MCF-7 parental cells [51]. In a urethane-induced lung carcinogenesis murine model, administration of