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REVIEW ARTICLE

The Role of Cucurbitacins in Combating Cancers: A Mechanistic Review

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ABSTRACT

Cucurbitacins are highly oxidized tetracyclic triterpenoids from the Cucurbitaceae families. Several cucurbitacins, such as B, D, E, I, R, IIa, and dihydrocucurbitacin B, have been shown to possess antiproliferative and anticancer activities. Mechanistically, cucurbitacins induce cell cycle arrest at the G2/M phase and induce apoptosis through several mechanisms, such as the production of reactive oxygen species. In addition, they can inhibit the migration and invasion of cancer cells. Consistently, cucurbitacins have been shown to inhibit the Janus kinase-mediated activation of the transcription factor, signal transducer and activator of transcription. In addition, other receptor-mediated signaling pathways, such as ErbB, human epidermal growth factor receptor 2, and integrins, have also been inhibited by the cucurbitacin on cancer cells. Cucurbitacin treatments for various types of cancer have been shown to disrupt the cytoskeletal components such as actin, inhibit the expression of the proto-oncogenic proteins such as c-myc, and induce the expression of tumor suppressor proteins such as p53. Importantly, the synergistic anticancer activities of cucurbitacins have been observed when combined with established chemotherapeutic drugs, such as imatinib mesylate, paclitaxel, docetaxel, and gemcitabine. Cucurbitacins are a promising anticancer agent and can potentiate the effect of current chemotherapy drugs as well as reduce the serious side effects of these drugs.

Key words: Apoptosis, cancer, cell cycle arrest, cucurbitacin, molecular mechanism, p53

INTRODUCTION

Cucurbitacins are highly oxidized tetracyclic triterpenoids. They are divided into 12 classes with over 200 derivatives and have a signature tetracyclic cucurbitacin nucleus with oxygen-containing groups in various positions.[1] At least 200 derivatives of cucurbitacins are available, from approximately 30 genera of Cucurbitaceae families.[2] In general, the various cucurbitacins are designated by letters, for example, cucurbitacins B, D, or E, but some of them are named with reference to their chemical structure or modification, for example, 3-epi-cucurbitacin F or 23,24-dihydrocucurbitacin B. In plants, cucurbitacins occur in the form of glycosides or free aglycones, with the most common being cucurbitacin B.[3] Cucurbitacins possess a broad range of pharmacological properties,[4] with great interest being given to their potent antiproliferative and anticancer activities.[5] Although the majority of anticancer activities are focused on cucurbitacins I and B, several researchers have reported on the anticancer activities of cucurbitacins D, E, R, IIa, and dihydrocucurbitacin B as well as a mixture of the glycosides of cucurbitacins B and E.[6,7] Cucurbitacins have been shown to exhibit anticancer activity both *in vitro* and *in vivo* through promoting cell cycle arrest, inhibition of migration, invasion, and initiation of apoptosis. Recently, several studies have demonstrated synergistic anticancer activities when cucurbitacins are combined with clinically approved chemotherapeutic drugs, such as imatinib mesylate, paclitaxel, docetaxel, and gemcitabine.

Therefore, cucurbitacins are a class of promising anticancer drugs that can be used alone or given with other chemotherapies or radiotherapies to treat many types of cancers [Figure 1]. In this review, we will provide a detailed summary of the research progress on the anticancer characteristics of various cucurbitacins and their molecular mechanisms against various cancers [Figure 1]. Although cucurbitacins are shown to be effective against many types of cancer, this review will focus on the molecular mechanisms of cucurbitacins against breast, colon, and lung cancers, leukemia, melanoma, hepatocellular carcinoma, ovarian cancer, and pancreatic cancer. This review would be particularly helpful for physicians who are currently treating patients with cancer and for scientists who are interested in elucidating the molecular mechanisms of anticancer activities.

CUCURBITACINS' EFFECTS ON BREAST CANCER

BRCA1 is a human tumor suppressor gene responsible for repairing DNA. BRCA1 is identified as a biomarker for breast cancer, with the lack of BRCA1 causing deficient DNA repair results and increased sensitivity to DNA damage-based chemotherapeutics. The presence of BRCA1 promotes sensitivity to antimicrotubule agents, most likely through the modulation of the cell cycle and apoptosis.[8] BRCA1 promotes the

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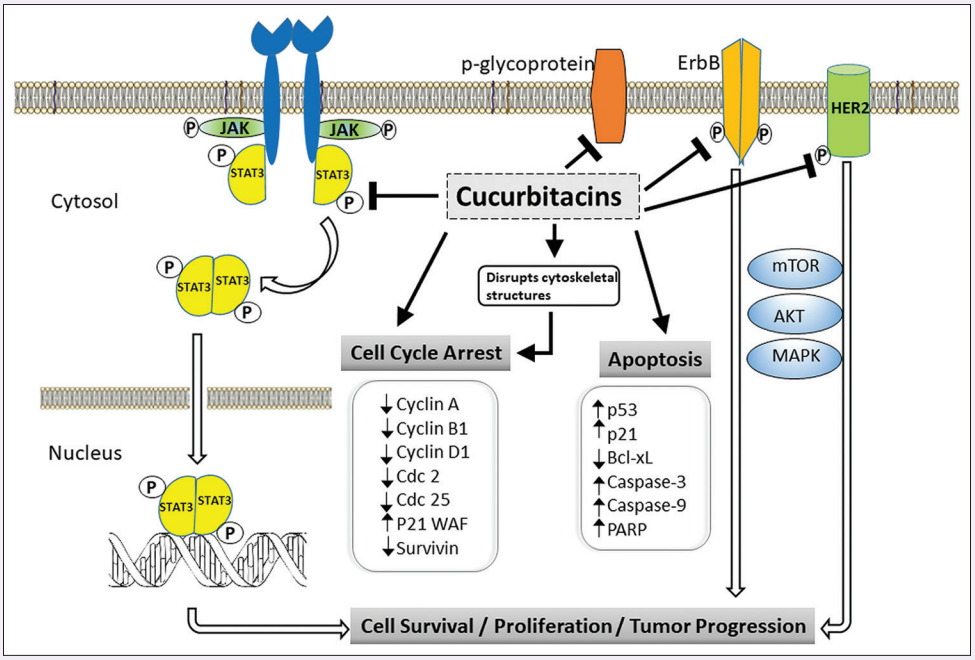

Figure 1: The mechanism of anticancer activities of cucurbitacins. Cucurbitacins are known to arrest the cell cycle and inhibit the growth of various cancer cell types by downregulation of cyclin-A, cyclin-B1, cyclin-D1, cdc-2, and cdc-25 and through the upregulation of p21 WAF. Cucurbitacins are known to induce apoptosis in several cancer types by inducing p53, p21, caspase-3, caspase-9, and poly ADP-ribose polymerase and by the decreasing the expression of Bcl-xL. Cucurbitacins are well known for the inhibition of signal transducer and activator of transcription pathways, leading to the inhibition of tumor cell survival or proliferation. Several cucurbitacins disrupt the cytoskeletal components, such as actin, and leading to cell cycle arrest. Cucurbitacins inhibit the activation of Erb2 and human epidermal growth factor receptor 2 receptors, leading to the inhibition of mTOR/AKT/mitogen-activated protein kinase signaling pathways and ultimately affecting the cancer cell survival, proliferation, and the tumor cell progression. Cucurbitacins also inhibit the expression of p-glycoprotein, an efflux pump, leading to the increased sensitivity of several chemotherapeutic agents

expression of the cell cycle checkpoint proteins, p21/Waf1 and p27Kip1, and inhibits the expression of the antiapoptotic protein survivin.[9] Loss of BRCA1 expression leads to an increase in survivin expression, resulting in reduced sensitivity to the chemotherapeutic drug, paclitaxel.[9] Promkan *et al.*[10] showed that cucurbitacin B, extracted from *Trichosanthes cucumerina L*, inhibited cellular proliferation, migration, invasion, and anchorage-independent growth in BRCA1 defective breast cancer cells, where the overexpression of wild-type BRCA1 significantly reduced the effectiveness of cucurbitacin B. Furthermore, cucurbitacin B promoted the expression of p21/Waf1 and p27Kip1 but inhibited the expression of survivin. This study indicates that survivin could be a vital target of cucurbitacin B in BRCA1 defective breast cancer cells.

A study by Bakar[11] showed that treatment with cucurbitacin B enhanced the chemosensitivity to imatinib mesylate, i.e., a combination of synergistically inhibited cell proliferation and induced apoptosis in MCF-7 tumor cell lines. Furthermore, it found that cucurbitacin B increased the inhibitory effect of imatinib mesylate on matrix metalloproteinase (MMP)-2 expression. On the other hand, Aribi *et al.*[12] reported that a combination of cucurbitacin B, with either docetaxel or gemcitabine, synergistically inhibited the proliferation of MDA-MB-231 breast cancer cells *in vitro* through increased apoptotic rates. Furthermore, this study showed that low-dose cucurbitacin B, in combination with either docetaxel or gemcitabine, significantly reduced tumor volume, as compared with monotherapy in the *in vivo* treatment of human breast cancer orthotopic xenografts in immunodeficient mice.[12]

Duangmano *et al.*[13] demonstrated the strong antiproliferative effects of cucurbitacin B against breast cancer cells MCF-7 and MDA-MB-231 by prominently altering the cytoskeletal network of breast cancer cells, inducing the alteration of morphology and the improper polymerization of the microtubule network. The study's results further showed that treatment with cucurbitacin B decreased the expression of c-myc and nucleophosmin/B23, where cucurbitacin B induced the translocation of nucleophosmin/B23 from the nucleolus to the nucleoplasm. This resulted in cell cycle arrest at the G2/M phase and the enhancement of apoptosis in breast cancer cells.[13] Previously, Duangmano *et al.* indicated that cucurbitacin B exerted an anticancer effect by inhibiting telomerase through downregulation of the human telomerase reverse transcriptase and c-myc expression in breast cancer cells T47D, SKBR-3, and MCF-7.[14] Sinha *et al.* reported that treatment with cucurbitacin B significantly inhibited the migratory and invasive potential of highly metastatic breast cancer cells MDA-MB-231 and 4T1; in this case, cucurbitacin B significantly inhibited the vascular endothelial growth factor (VEGF)-induced phosphorylation of focal adhesion kinase and MMP-9 signaling.[15]

Cucurbitacin B has also been shown to reduce the cell viability of MCF-7 breast cancer cells by inducing DNA damage, evidenced by the long tails in the comet assay and an increase in the γH2AX protein expression.[16] In this study, it was found that treatment with cucurbitacin B increased the production of reactive oxygen species and also induced autophagy, as evidenced by monodansylcadaverine staining and autophagic protein expression.[16] However, Dakeng *et al.* showed that cucurbitacin B induced apoptosis and exerted a growth inhibitory effect in breast cancer cell lines SKBR-3 and MCF-7 through interruption of the Wnt signaling by inhibiting the translocation of β-catenin and galectin-3 into the nucleus.[17]

Human epidermal growth factor receptor 2 (HER2)/neu is a proto-oncogenic receptor and is overexpressed in 25%–30% of breast cancer patients,[18] while integrins are a large family of cell adhesion

receptors, which also perform other diverse functions such as cell survival and motility.[19,20] A study by Gupta and Srivastava[21] found that cucurbitacin B induced apoptosis in breast cancer cells through inhibition of HER2; this correlates with the suppression of integrin-α6 and integrin-β4, which are overexpressed in breast cancer cells.

In general, cucurbitacins are considered to be selective inhibitors of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways.[5,22,23] JAK is a cytoplasmic tyrosine kinase, and when it is phosphorylated, it can activate the transcription factor STAT. The activation of the JAK/STAT signaling pathway results in the expression of genes involved in immunity, proliferation, oncogenesis, apoptosis, and differentiation.[24] A study by Lan *et al.*[25] showed that cucurbitacin E inhibited the growth of human breast cancer cell lines Bcap-37 and MDA-MB-231 *in vitro* by inducing the G2/M phase cell cycle arrest and cell apoptosis through the activation of caspase-3, upregulation of the expression of p21 and p27, and inhibition of the activation of STAT3. Furthermore, treatment with cucurbitacin E potentiated the effect of cisplatin on breast cancer cells. Although other mechanisms, such as the activation of the mitogen-activated protein kinase (MAPK) pathway, are also known to be important for cancer cell proliferation and survival, the Lan *et al.*'s study[25] found that only STAT3 signaling, but not the MAPK pathway, was impaired in breast cancer cells treated with cucurbitacin E.

Estrogen receptor-alpha (ERα), progesterone receptor (PR), and HER2 triple-negative breast cancer accounts for 15%–25% of breast tumors[26] and is a highly aggressive form of breast cancer resistant to many common treatments. Kong *et al.*[27] compared the effects of 12 different cucurbitacins on triple-negative breast cancer type and found that cucurbitacin E was the most potent cytotoxic compound, demonstrating decreased cell viability in various triple-negative breast cancer cell lines. In these cases, cucurbitacin E induced G2/M cell cycle arrest and apoptosis in the MDA-MB-468 and SW527 TNBC cell lines. It was noted that cucurbitacin E downregulated the expression of cyclin D1, survivin, XIAP, Bcl-2, and Mcl-1; inactivated STAT3, AKT, and extracellular-regulated kinase (ERK); and activated c-Jun N-terminal kinase (JNK).[27]

ErbB receptors are a family of tyrosine kinases comprising of four members (ErbB1, ErbB2, ErbB3, and ErbB4). They play multiple roles in cancer cell proliferation, survival, and motility.[28] Hyperactivation of the ErbB receptors is associated with the progression of some human cancers, including breast, prostate, and lung cancers. Overexpression of ErbB2 receptors is often associated with aggressive tumor behavior and poor prognosis, while hyperactivation of ErbB3 is common in breast cancer.[28] Rho GTPases are well-established effectors of ErbB receptors. Rac1, a member of the Rho family, has been widely implicated in breast cancer cell migration and proliferation, in response to ErbB ligands.[29] Lopez-Haber and Kazanietz[30] indicated that cucurbitacin I inhibited breast cancer cell motility and Rac1 activation by ErbB receptors. Furthermore, this study reported that ErbB-driven Rac1 activation in breast cancer cells proceeds independently of the JAK2/STAT3 pathway. In addition, the inhibitory effect of cucurbitacin I on Rac1 activity involves the disruption of the balance between RhoA and Rac1.[30] Thus, cucurbitacin I can inhibit Rac1 activation in breast cancer cells, independent of the JAK2 pathway,[30] indicating the presence of other mechanisms for the tumor suppressive function of cucurbitacins.

Cucurbitacin I has also been shown to inhibit tumor angiogenesis in the human breast cancer cell line MDA-MB-468 *in vitro* through the reduction of STAT3 phosphorylation and the reduction of VEGF transcription and secretion. This suggests that cucurbitacin I is involved in the inhibition of the VEGF autocrine loop in the tumor microenvironment.[31]

Kim *et al.*[32] found that ethanolic extract of *T. kirilowii* and cucurbitacin D suppressed the proliferation and induced apoptosis and G2/M cell cycle arrest in MDA-MB-231 breast cancer cells. This was accomplished by inhibiting STAT3 phosphorylation, thus inhibiting the nuclear translocation and transcriptional activity of STAT3.

CUCURBITACINS' EFFECTS ON COLORECTAL CANCER

The use of cucurbitacin I on SW480 cells, human colon cancer cells, decreased cell viability and cell proliferation by inducing G2/M phase cell cycle arrest, with a decreased expression of cell cycle proteins, including cyclin B1, cyclin A, CDK1, and CDC25C.[33] Furthermore, treatment with cucurbitacin I induced the apoptosis of colorectal cancer cells by increasing the cleavage of caspase-3, caspase-7, caspase-8, caspase-9, and poly ADP-ribose polymerase (PARP). In addition, *in vivo* treatment with cucurbitacin E inhibited the tumorigenicity and growth of CT-26 cells in syngeneic BALB/c mice.[33] A study by Song *et al.*[34] reported that the exposure of the Colo-205 cells to cucurbitacin I significantly decreased cell viability as well as suppressed cell migration and the invasion of colon cancer. It was found that the anticancer activity of cucurbitacin I was attributed to the downregulation of p-STAT3 and MMP-9 expression.[34] The glucoside derivative of cucurbitacin I was shown here to be specifically and strongly cytotoxic against Caco-2 cells, a human colon cancer epithelial cell line.[35]

Hsu *et al.*[36] studied the anticancer effects of cucurbitacin E on colorectal cancer using primary cell lines isolated from five colorectal cancer patients. Findings demonstrated that cucurbitacin E inhibited tumor growth by arresting the cell cycle in the G2/M phase and delayed mitosis through the upregulation of GADD45-γ gene expression and the blockage of the cyclin B1/CDC2 complex. In the same study, it was observed that the inhibition of proliferation, the arresting of mitosis, the production of reactive oxygen species, and the loss of mitochondrial membrane potential ($\Delta\Psi m$) were found to be dependent on the quantity of cucurbitacin E used to treat the cancer cells.[36]

Bakar[11] reported that treatment with cucurbitacin B, along with imatinib mesylate, had a synergistic effect on the inhibition of cell proliferation and the induction of apoptosis on the decreased expression of MMP-2 in SW480 human colon cancer cells. Another study demonstrated that cucurbitacin B inhibited the growth of these cells by increasing the production of reactive oxygen species and by inducing G (2) phase arrest and apoptosis in a dose-dependent manner.[37] Although epidermal growth factor receptor (EGFR) and its downstream JAK/STAT3 pathways are shown to mediate the pathogenesis of colorectal cancer,[38] a study by Yasuda *et al.* found that the phosphorylation of STAT3 was not affected by cucurbitacin B in human colon cancer cells.[37] However, the Yar Saglam *et al.*'s study[39] indicated that cucurbitacin B induced cell cycle inhibition and apoptosis in HT-29 and HCT-116 colorectal cancer cells by inhibiting the STAT3 and EGFR signaling. The combination of cucurbitacin B with gefitinib, an orally active inhibitor targeting the adenosine triphosphate-binding domain of EGFR, was found to be more effective than cucurbitacin B alone in inducing apoptosis in colorectal cancer cells.[39]

Escandell *et al.*[40] studied the anticancer activity of cucurbitacins in colon cancer cell lines that did not harbor activated STAT3. This study found that cucurbitacins induced remarkable changes in the cytoskeleton structure of actin and tubulin microfilaments, inhibited the proliferation, and induced apoptosis in colon cancer cell lines HCT-116 and Hke-3. However, the presence of oncogenic kRas significantly decreased the sensitivity of the cells to cucurbitacin R, cucurbitacin I, and 23,24-dihydrocucurbitacin B. In human colon cancer cells HCT-116, which encompass mutant Ras, cucurbitacins induced the expression of p53 and p21. These results indicate that cucurbitacin is effective on human colon cancer cells in the absence of active kRas and STAT3.[40]

CUCURBITACINS' EFFECTS ON LEUKEMIA

Several types of cucurbitacins have been found effective against leukemia. Cucurbitacin I induced the suppression of serine 727 phosphorylation of STAT3, leading to apoptosis and cell cycle arrest through alterations in the gene transcription of B-leukemia cells.[41] It also activated the JNK signaling pathway, independent of apoptosis, and cell cycle arrest, leading to an increased VEGF expression in B-cell leukemia cells.[42] Treatment of murine lymphoma tumor cells with cucurbitacin I upregulated the TRAIL receptor which mediates cytotoxicity and growth inhibition, in addition to activating caspases.[43] Hira *et al.*[43] noted that the murine dendritic cells, upon stimulation with recombinant interleukin (IL)-15 *in vitro* or *in vivo*, express a tumor necrosis factor superfamily member TRAIL, which mediated the cytotoxicity and apoptosis. Naive peripheral blood dendritic cells derived from chronic myeloid leukemia patients exhibited significantly impaired expression of TRAIL, whereas priming tumor cells with recombinant IL-15 prolonged the survival of tumor-bearing mice treated with cucurbitacin I.

Cucurbitacin D was found to inhibit the proliferation, as well as to induce apoptosis, of T-cell leukemia cells. Moreover, the constitutively activated nuclear factor (NF)-κB was inhibited by cucurbitacin D in the nucleus, which resulted in the accumulation of NF-κB in the cytoplasm, leading to downregulation of the expression of antiapoptotic proteins such as Bcl-xL and Bcl-2. Further, cucurbitacin D potentiated the antiproliferative effects of the histone deacetylase inhibitor, valproic acid (VPA). *In vivo* studies using SCID mice have established the cucurbitacin D-mediated pro-apoptotic activity and the inhibition of proteasome activity.[44]

Cucurbitacin E induced apoptosis in HL-60 cells through activation of caspase-3, caspase-8, and caspase-9. In addition, it decreased the levels of the antiapoptotic proteins XIAP, survivin, and Mcl-1 but increased the level of the pro-apoptotic protein, Bax. Further, cucurbitacin E is shown to induce cell growth arrest and apoptosis by inhibiting protein translation through inducing the phosphorylation of translation initiation factor 2.[45] The cytotoxic effects of cucurbitacin E were mediated by the inhibition of the phosphorylation of cofilin, resulting in the depolymerization of actin in U937 human leukemia cells.[46]

Cucurbitacin B significantly decreased the cell viability of K562 leukemia cells in a concentration-dependent manner by causing DNA damage and arresting the cell cycle at the G2/M phase and inducing apoptosis through an increased level of intracellular reactive oxygen species.[47] Further, it was observed the induction of nuclear γH2AX foci, an increased intracellular calcium ion concentration, and depolarization of mitochondrial membrane potential in cucurbitacin B-treated K562 leukemia cells.[47] Leukemia cell growth was inhibited by cucurbitacin B through G2/M phase arrest and apoptosis as well as through inhibition of STAT3 and the Raf/MEK/ERK pathway in the K562 cells.[48] Treatment with cucurbitacin B showed a significant S-phase cell cycle arrest, enlarged cell size, multinucleation, and alteration of the cytoskeletal network of leukemic cells, inducing rapid and improper polymerization of the F-actin network in myeloid leukemic cells[49] and in human T-cell leukemia Jurkat cells.[50] Cucurbitacin B synergistically enhances the apoptosis-inducing effect of arsenic trioxide by inhibiting STAT3 phosphorylation in lymphoma Ramos cells.[51]

CUCURBITACINS' EFFECTS ON LUNG CANCER

Nonsmall cell lung cancer (NSCLC) is one of the most common malignant tumors, with a high mortality rate due to the elevated risk of resistance. Marostica *et al.* studied the effect of a semi-synthetic derivative of cucurbitacin B (2-deoxy-2-amine-cucurbitacin), for its *in vitro* synergistic anti-proliferative effects, when combined with paclitaxel in A549 adenocarcinomic human alveolar basal epithelial cells.[52] Further, they evaluated the *in vivo* antitumor efficacy of this combined therapy using a human NSCLC xenograft model. The combination therapy resulted in a significantly effective reduction of the relative tumor mass and tumor volume, as well as the highest inhibition of tumor growth and proliferation, when compared with the individual treatments.[53] In a separate study, D2-deoxy-2-amine-cucurbitacin was shown to arrest the cell cycle of lung epithelial cells at the G2/M phase and induced cell apoptosis by interfering with the activation of EGFR and its downstream signaling, including AKT, ERK, and STAT3.[54] Treatment with cucurbitacin B decreased the cell viability of EGFR-wild-type (A549 and H1792) and EGFR-mutant lung cancer cells (H1650 and H1975) through inhibition of PI3K/mTOR and STAT-3 signaling, along with the activation of adenosine monophosphate (AMP)-activated protein kinase-α.[55]

The cucurbitacin B-mediated inhibition of DNA methyltransferase and histone deacetylase in lung cancer NSCLC H1299 cells resulted in the reactivation of key tumor suppressor genes, such as CDKN1A and CDKN2A, in the downregulation of oncogenes c-myc and kRas, and in the key tumor promoter gene, human telomerase reverse transcriptase. This led to the inhibition of cellular proliferation and the induction of cellular apoptosis in NSCLC. Moreover, cucurbitacin B treatment significantly inhibited the tumor incidence and the hyperproliferation in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice.[56] It dose dependently inhibited A549 lung cancer cell proliferation by inhibition of the cell cycle and downregulation of cyclin B1 and the induction of apoptosis associated with the release of cytochrome c, downregulation of B-cell lymphoma 2, and inhibition of the STAT3 pathway.[57] Cucurbitacin B-induced DNA damage in A549 cells was mediated by an increased intracellular reactive oxygen species formation, leading to G2/M cell phase arrest through ataxia telangiectasia-mutated-activated Chk1-Cdc25C-Cdk1 and p53-14-3-3-σ signaling.[58] The sensitivity of benzo (a) pyrene-trans-7,8-dihydrodiol-9,10-epoxide [BPDE]-transformed human 16HBE bronchial epithelial cells (16HBE/BPDE cells) to p53-specific cytotoxic T-lymphocytes was enhanced by treatment with cucurbitacin B through inhibition of JAK2/STAT3 activation and also by activation of the interferon-γ-related STAT1 activation.[59] Furthermore, this form of cucurbitacin significantly reduced the frequency of immature myeloid cells in patients with lung cancers and enhanced the effect of p53-specific cytotoxic T-lymphocytes on tumor 16HBE/BPDE cells.[59]

Cucurbitacin I has been reported to inhibit the cell growth of human NSCLC through inhibition of the PI3K/AKT/p70S6K signaling pathway.[60] In addition, it dose dependently inhibited the phosphorylation of STAT3 but enhanced the phosphorylation of STAT1 in lung adenocarcinoma A549 cells. Treatment with cucurbitacin I also disrupted the actin filaments physically associated with JAK2 and STAT3, and with STAT1 in A549 cells.[61] The cancer cell stemness gene CD133-positive NSCLC cells were also inhibited, which facilitated the differentiation of CD133-positive NSCLC cells into CD133-negative NSCLC cells. Moreover, treatment with cucurbitacin I enhanced the chemo-radiotherapy response and improved the survival of NSCLC-CD133-positive-transplanted, immunocompromised mice.[62]

Cucurbitacin E induced reactive oxygen species-dependent apoptosis in 95D lung cancer cells. In addition, treatment with this type of cucurbitacin damaged the F-actin without affecting β-tubulin and induced protective autophagy mediated through reactive oxygen species through activation of the AKT/mTOR pathway in lung cancer cells.[63] Cucurbitacin A induced G2/M cell cycle arrest in A-549 NSCLC cells through inhibition of the mTOR/PI3K/AKT signaling pathway.[64] Treatment with cucurbitacin-D-induced cyclin-dependent

kinase 1 mRNA upregulated and blocked the G1 phase of cell cycle proliferation and induced apoptotic cell death in the nonsmall cell lung carcinoma cell line.[65]

CUCURBITACINS' EFFECTS ON PROSTATE CANCER

Prostate cancer is a common cancer in males, mostly occurring between 60 and 70 years. Cucurbitacin B significantly and specifically inhibited prostate cancer cell growth in PC-3 and LNCaP human prostate cancer cell lines and induced apoptosis.[66] The pretreatment of mice with cucurbitacin B 2 weeks before PC-3 prostate cancer cell implantation significantly reduced the rate of *in vivo* tumor formation by day 31, compared with the respective controls. Mechanistically, cucurbitacin B inhibited the phosphorylation and activation of an important enzyme for cancer metabolism, ATP citrate lyase, in both *in vitro* and *in vivo* tumor model experiments. This study indicates that the anticancer activity of cucurbitacin B against prostate cancer is mainly through the inhibition of the signaling of the enzyme ATP citrate lyase.[66]

A derivative of the cucurbitacin family, 23,24-dihydrocucurbitacin F, has been isolated as an active component from the root of *Hemsleya amabilis*.[67] The effect of 23,24-dihydrocucurbitacin F was tested on human prostate cancer cell lines DU145, PC3, and LNCaP. The results showed that this cucurbitacin derivative inhibited cell growth and induced cell cycle arrest at the G (2)/M phase, inhibited the formation of binucleated cells and increased the levels of apoptosis in all prostate cancer cell lines tested. After treatment with 23,24-dihydrocucurbitacin F, G-actin depletion, researchers observed actin aggregation and rod-like actin fibers, with little effect on microtubule structure. The formation of actin aggregates and further the cofilin–actin rod formation were associated with the dephosphorylation of cofilin-1 (cofilin) that lead to cell cycle arrest, cytokinesis failure, and apoptosis.[67]

Cucurbitacin E significantly reduced the cell viability and induced apoptosis in the LNCaP prostate cancer cell line. In addition, these effects were mediated through the activation of cofilin-1, p-mTOR, AMP-activated protein kinase, cellular tumor antigen p53, and caspase-9 protein expression in LNCaP cells.[68] Moreover, cucurbitacin E was shown to disrupt the cytoskeletal integrity by disrupting the actin and vimentin cytoskeleton in prostate carcinoma cells.[43]

CUCURBITACINS' EFFECTS ON MELANOMA

The effects of cucurbitacins have been tested against various melanoma cell lines, such as A-375, Sk-Mel-28, and mutant B-Raf. Cucurbitacins have been shown to improve activity against human skin malignant melanoma cell lines A-375 and Sk-Mel-28 through inhibition of the total and phosphorylated ERK.[6] Since several cucurbitacins have been shown to disrupt the actin cytoskeleton structure in various cancer cell types, Zhang *et al.*[69] studied the molecular mechanism of the cucurbitacin B-induced disruption of the actin cytoskeleton in melanoma cells. They found that cucurbitacin B induced actin aggregation, followed by the formation of rod-like structures of actin in melanoma cells.[69] Furthermore, they reported that this form of cucurbitacin activated cofilin, a key regulator in actin cytoskeletal dynamics, by its dephosphorylation. The activated cofilin then forms rod-like aggregates, which bind with actin rods. The hyperactivation of cofilin and the cofilin–actin rod formation were downstream events of cucurbitacin B-induced actin aggregation.[69] Furthermore, cucurbitacin B-induced cofilin hyperactivation was dependent on the cofilin phosphatase slingshot homolog 1 but was independent of the cofilin phosphatase chronophin.[69]

In addition, cucurbitacin B treatment rapidly induced the activation of vasodilator-stimulated phosphoprotein (VASP) through phosphorylation at the Ser157 residue, and this resulted in the generation of VASP clumps.[70] These VASP clumps bind with amorphous actin and form highly ordered cofilin–actin rods in melanoma cells.[70] The cucurbitacin B-induced VASP activation, actin aggregation, and cofilin–actin rod formation were shown to be mediated by cAMP-independent PKA activation and by the activation of Gα13 and RhoA.[70]

Ouyang *et al.*[71] found that cucurbitacin B inhibited the proliferation of B16F10, a mouse melanoma cell line, in a dose-dependent manner, and simultaneously, a pro-survival compensatory response was induced, involving autophagy and the upregulation of antiapoptotic protein Bcl-2. In support of this, the inhibition of autophagy using chloroquine increased the cytotoxicity of cucurbitacin B in melanoma cells, and this autophagy was mediated through a transient activation of JNK.[71] Importantly, when cucurbitacin B was combined with VPA, an inhibitor of histone deacetylase, a synergistic cytotoxicity was found through induction of apoptosis in a prolonged activation of the JNK pathway in the B16F10 melanoma cell line.[71]

Cucurbitacin B exhibited strong inhibitory effects on cell proliferation and colony formation, as well as the migration and invasion potential of murine B16F10 melanoma cells.[72] Mechanistically, it was observed that treatment of melanoma cells with cucurbitacin B induced G (2)/M-phase arrest and a rapid cell membrane blebbing, deformation, and formation of multiploid cells.[72] The *in vivo* growth of subcutaneous melanoma was significantly inhibited in mice treated with cucurbitacin B. The antitumor activity of this form of cucurbitacin was partly explained by a rapid depletion of the G-actin pool and formation of actin aggregates in a reactive oxygen species-dependent manner in melanoma cells.[72]

Cucurbitacin D and 23,24-dihydro-cucurbitacin D, isolated from the root of *T. kirilowii*, effectively inhibited the synthesis of melanin in B16/F10 melanoma cells through the inhibition of a tyrosinase enzyme, hydroxylates tyrosine, as the first step in the synthesis of melanin.[73] Knecht *et al.*[74] reported that cucurbitacin I inhibited the cell motility of B16-F1 melanoma cells and caused the aggregation of actin.

CUCURBITACINS' EFFECTS ON HEPATOCELLULAR CARCINOMA

Chan *et al.*[75] explored the anticancer activities of cucurbitacin B in hepatocellular carcinoma using the BEL-7402 cell line. Treatment with this type of cucurbitacin induced S phase arrest and apoptosis in the hepatocellular carcinoma cell line. This growth arrest was mediated by the downregulations of cyclin D1 and cdc-2 and through the inhibition of c-Raf activation but without affecting STAT3 phosphorylation.[75] Moreover, the *in vivo* anticancer activity of cucurbitacin B against hepatocellular carcinoma was shown through its effective inhibition of the xenograft of BEL-7402.[75]

Cucurbitacin B has been found to inhibit HepG2 cell viability in a dose- and time-dependent manner, resulting in the accumulation of HepG2 cells at the S phase and induction of apoptosis, which was clearly evidenced by the condensation of chromatin, nuclear fragmentation, and the presence of apoptotic bodies.[76] Protein analysis showed that cucurbitacin B treatment inhibited the phosphorylation of STAT3 and also downregulated the expression of Bcl-2. Furthermore, the *in vivo* anticancer activity of cucurbitacin B was evidenced by the inhibition of the growth of HepG2 tumor in nude mice.[76]

Sun *et al.*[77] studied the combination of cucurbitacin B and curcumin, noting enhanced apoptosis induction and reversal of multidrug resistance in human hepatoma cells, BEL7402/5-Fu cells *in vitro* and BEL7402 tumor-bearing mice *in vivo*. Mechanistically, it was found that cucurbitacin B treatment reduced the expression of P-glycoprotein, also known as multidrug resistance protein 1, which is an efflux pump

that pumps numerous foreign substances out of the BEL7402/5-Fu cells.[77] Cucurbitacin B induced DNA damage, apoptosis, and protective autophagy mediated by reactive oxygen species in hepatocellular carcinoma cells. The activation of the tumor suppressor protein phosphatase and tensin homolog by DNA damage induced protective autophagy in response to cucurbitacin B treatment.[78]

Ayyad *et al.*[79] isolated cucurbitacin E and cucurbitacin I from *Citrullus colocynthis* grown in Saudi Arabia. These exhibited a potent *in vitro* cytotoxic activity against hepatoma cell line HepG2. Two other studies demonstrated that cucurbitacin D and cucurbitacin J exhibited strong growth inhibitory activities against hepatocellular carcinoma BEL-7402 cells *in vitro*.[80,81] Takahashi *et al.*[82] isolated cucurbitacin D from *T. kirilowii*, and this form induced apoptosis in hepatocellular carcinoma cells through activation of caspase-3 and phosphorylation of JNK *in vitro*.

Hepatic stellate cells that are activated by human hepatocellular carcinoma cells secrete a wide variety of cytokines, which are mainly responsible for the microenvironment of hepatocellular carcinoma. Cui *et al.*[83] showed that the combination of 8-bromo-7-methoxychrysin and cucurbitacin I synergistically suppressed the characteristics of liver cancer stem-like cells, which have the capacity of sustaining human hepatocellular carcinoma self-renewal and progression in the human hepatocellular carcinoma cell line SMMC-7721 cells.

On the other hand, cucurbitacin E was shown to have a strong hepatotoxicity through a potent inhibition of both CYP3A and P-glycoprotein activities *in vitro*.[84] However, *in vivo* animal studies show that acute treatment with cucurbitacin E inhibited CYP3A and P-glycoprotein, while chronic treatment with the same substance resulted in the induction of CYP3A and P-glycoprotein.[84] These results show that cucurbitacin E may cause complex drug-drug interactions.

CUCURBITACINS' EFFECTS ON OVARIAN CANCER

Ishii *et al.*[85] found that cucurbitacin D inhibited the growth of endometrial and ovarian cancer cell lines. In that study, cell cycle analysis showed that cucurbitacin D increased the proportion of cells in sub-G0/G1 phases and G2/M phases of the cell cycle and induced apoptosis in endometrial and ovarian cancer cell lines.[85] Furthermore, the altered expression of genes related to cell growth, malignant phenotype, and apoptosis was in accordance with cell cycle arrest and induction of apoptosis.[85]

Cucurbitacin E was reported to increase the concentration of doxorubicin in M5076 ovarian sarcoma by suppressing the efflux of doxorubicin *in vitro*, resulting in the maintenance of the doxorubicin level in tumor cells.[86,87] Although the mechanism is unclear, it was observed that the co-treatment of cucurbitacin E significantly increased the concentration of doxorubicin in the tumor but decreased the concentration of doxorubicin in normal tissues. The *in vivo* experiments showed that treatment with doxorubicin alone did not alter the tumor size or tumor weight of M5076 ovarian sarcoma, but the combination of cucurbitacin E with doxorubicin resulted in decreased tumor size and tumor weight.[86,87] These results clearly indicate that the combination of cucurbitacin E with doxorubicin may be an effective strategy in combating cancer chemotherapy in general, ovarian cancer specifically.

El-Senduny *et al.*[88] studied the effect of cucurbitacin B on cisplatin-resistant ovarian cancer cells. The pretreatment of cisplatin-resistant ovarian cancer cells A2780CP with cucurbitacin B led to a significant increase in the cytotoxicity of cisplatin. The cucurbitacin B-induced sensitization effect of cisplatin was partly dependent on the depletion of the total glutathione and an increase in the level of reactive oxygen species and through a decrease in the level of dual-specificity tyrosine-regulated kinase (Dyrk1B), pERK1/2, and pSTAT3 level.[88] Treatment with a combination of cisplatin and cucurbitacin B significantly decreased the viability of 3D spheroids developed from the ovarian cancer cell line A2780CP.[88] The results of this study have demonstrated that cucurbitacin B is an effective chemosensitizer for cisplatin-resistant ovarian cancer.

Cucurbitacin B was reported to exert a dose- and time-dependent cytotoxicity against the ovarian cancer A2780 cell line.[89] Treatment with cucurbitacin B induced cell cycle arrest at the G2/M phase of the cell cycle in the sensitive A2780 and the paclitaxel-resistant A2780/Taxol cells.[89] Cucurbitacin B-induced apoptosis was accompanied by the activation of caspase-3, the downregulation of B-cell lymphoma-2, and an enhanced expression of p53 and p21 in the two cell lines.[89] Protein analysis showed that cucurbitacin B enhanced the expression of p53 and p21 in the sensitive A2780 and the paclitaxel-resistant A2780/Taxol cells.[89] Furthermore, cucurbitacin B also caused a downregulation of the expression of P-glycoprotein, an efflux pump.[89]

Liu *et al.*[90] showed that cucurbitacin A is an effective anticancer treatment against ovarian cancer cell line SKVO3, through inducing DNA damage and reactive oxygen species-mediated alterations in mitochondrial membrane potential. Treatment with cucurbitacin A also triggered cell cycle arrest at the G2/M checkpoint and inhibited the expression of the PI3K/AKT/mTOR signaling pathway in SKVO3 cells.[90]

CUCURBITACINS' EFFECTS ON PANCREATIC CANCER

Sun *et al.*[91] showed that cucurbitacin E exhibited a dose- and time-dependent inhibition of the growth of human pancreatic cancer cells PANC-1 by arresting the growth at the G (2)/M phase and inducing apoptosis. Mechanistically, treatment with cucurbitacin E inhibited the phosphorylation of STAT3 and at the same time enhanced the expression of p53.[91] Furthermore, it was found that treatment with cucurbitacin B activated caspase-3, increased the expression of p21, and decreased the expression of Bcl-2 and survivin.[92]

Iwanski *et al.*[93] examined the *in vivo* antitumor effects and the toxicities of the combination of cucurbitacin B and gemcitabine, an anticancer agent used in various chemotherapies, against a murine xenograft model of human pancreatic cancer cells. This combination therapy resulted in a highly significant (up to 79%) growth inhibition of pancreatic cancer xenografts and importantly low toxicity. The protein analysis of the mice tumor samples that received the combination therapy exhibited a stronger inhibition of Bcl-XL, Bcl-2, and c-myc and an enhanced activation of the caspase cascades.[93]

Thoennissen *et al.*[23] reported that the cucurbitacin B-induced G (2)-M-phase arrest and apoptosis in human pancreatic cancer cells were associated with the inhibition of activated JAK2, STAT3, and STAT5. The *in vivo* and *in vitro* experiments on human pancreatic cancer cells demonstrated that treatment with cucurbitacin B increased the level of p21 (WAF1), decreased the expressions of cyclin A, cyclin B1, and Bcl-XL, and ultimately resulted in the activation of the caspase cascade.[23] Significantly, the combination therapy of cucurbitacin B and gemcitabine synergistically decreased the volume of pancreatic tumor xenografts in athymic nude mice.[23]

Treatment with cucurbitacin B was shown to inhibit the proliferation of pancreatic cancer cells by arresting them at the G2/M cell cycle and by inhibiting the expression of EGFR and its downstream signaling pathways, such as PI3K/AKT/mTOR and STAT3.[94] Furthermore, the combination therapy of cucurbitacin B and an ERK inhibitor significantly inhibited the signaling of EGFR, PI3K/AKT/mTOR, STAT3, and ERK.[94] Moreover, this combination treatment enhanced the pro-apoptotic protein Bim

and decreased the antiapoptotic proteins Mcl-1, Bcl-2, Bcl-xL, and survivin.[94] The *in vivo* effect of the combination therapy resulted in a highly significant growth inhibition of pancreatic cancer xenografts.[94]

CONCLUSION

Several *in vitro* studies have clearly documented the potent effects of cucurbitacins on arresting cancer cell growth and inducing apoptosis, while other studies have shown that cucurbitacins can disrupt cancer's structural components, such as actin, and inhibit the migration and invasion of cancer cells [Figure 1]. Although several cucurbitacins, such as B, D, E, I, and R, have been studied for their anticancer activities, cucurbitacins B, E, and I have been broadly reported. Mechanistically, cucurbitacins have been promising in their ability to induce cell cycle arrest at the G2/M phase and induce apoptosis through several mechanisms, such as the production of reactive oxygen species. A few studies have shown the cucurbitacin-mediated activation of autophagy in cancer cells. Cucurbitacins have been consistently shown to inhibit the JAK/STAT and receptor-mediated signaling pathways, such as ErbB, HER2, and integrin-α6, in cancer cells. Other anticancer mechanisms, such as inhibiting the expression of proto-oncogenic proteins (c-myc) and inducing the expression of a tumor suppressor protein (p53), have been supported by treatment with cucurbitacins. Notably, several studies have demonstrated synergistic anticancer activities when cucurbitacins are combined with clinically approved chemotherapeutic drugs, such as imatinib mesylate, paclitaxel, docetaxel, and gemcitabine. Although numerous *in vitro* and *in vivo* effects of cucurbitacins have been documented, studies of *in vivo* genetic cancer mouse models are necessary to take cucurbitacins to the next level, i.e., clinical trials, in order to treat human patients. Based on the current accumulation of evidence, the promising anticancer agent cucurbitacin can potentiate the effect of current chemotherapy drugs and in addition reduce the serious side effects associated with these drugs.

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Conflicts of interest

There are no conflicts of interest.

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