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Review Article

Cucurbitacins: A Focus on Cucurbitacin E As A Natural Product and Their Biological Activities

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Abstract

For the last years, different types of cucurbitacins have been extracted from various species of Cucurbitaceae family. For this review, all related papers were accumulated by searching electronic databases in the English language, including PubMed, Scopus, and Google Scholar. The keywords of cucurbitacin, cucumber anticancer therapy, cytotoxic effects, chemotherapy, and inhibitor effect were searched until February 2020. According to the result of this review, cucurbitacin E as a tetracyclic triterpenes compound, has been exhibited cell cycle arrest, anti-inflammatory and anticancer activities. It showed tumor proliferation prevention, induction of apoptosis or synergistically acts with other established antitumor compounds and cytokines throughout many molecular mechanisms. In a function-structure association manner, cucurbitacin E can inhibit Janus kinas2 (JAK2) phosphorylation, the signal transducer activator of transcription 3 (STAT3) and subsequently block these pathways, which seems to be the main mechanism of its activity. Future studies could target its detection in uninvestigated sources, subsequently its derivatives to improve their anticancer activity.

Introduction

Cancer causes 12 % mortality in the worldwide. Surgery and radiotherapy as well as chemotherapy are the most prevalent treatments for cancer.[1] Due to the limitations of chemotherapy such as side effects, toxicity, drug resistance and lack of specificity toward tumor cells leads to more cancer related deaths.[1,2] Many plant-derived compounds have antitumor activity. The anti-cancer activity of Cucurbitacin (Cu) has also attracted the attention of scientists.[3-7] Cu is a oxygenated tetracyclic triterpenoid, generally detected in the cucurbitaceae family that is recognized with bitterness and/or toxicity.[8-10] Structurally, Cu is categorized by the tetracyclic cucurbitane skeleton, with oxygen substitutions at different positions. Based on their structures, Cus are categorized into twelve compounds. Among the 17 main Cu (A to T), Cu B, D, E, I, and their derivatives showed strong antitumor activities and Cu F, O, P, Q, and their derivatives showed moderate anticancer activities.[5,11] Bioactive compound functionalization is the most powerful method for novel drug discovery.[12] Cu have been exhibited anti-proliferative effect on the several human cancer cell lines and various tumors such as "prostate, breast, uterine, lung, skin, liver, ovarian, brain, colon, leukemia, melanoma, renal, pharynx, pancreatic, nasopharyngeal and numerous other carcinomas".[2,3,13]

The inhibitory effects of Cu on cancer cells are through many molecular mechanisms including inhibition of proliferation, induction of apoptosis, suppression of tumor angiogenesis through interaction with vascular-endothelial-growth-factor-receptor-2-(VEGFR2-) intermediated JAK2-signal transducer then STAT3 and other pathways.[2] Cu also showed synergistic effect with known chemotherapeutic agents.[5,6]

Cucurbitacin E (CuE, a-elaterin), is a biological active chemical component from traditional Chinese medicine, extracted from *Cucubita pepo cv Dayangua*. Previous researches has shown that CuE disrupts cell actin and so suppressed cell adhesion

CuE has recently been reported to have prophylactic effects on cancer cell proliferation, permeability, and actin polymerization. However, some CuE activity is still unknown, such as its inhibitory effect on tumor angiogenesis.[14] Nevertheless, it is well suggested to verify toxicity of these compounds in vivo earlier to their utilization. Since Cu is

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very toxic, few papers have deal with their pharmaceutical activity. Therefore, in the present study, some details of the biological activities and anti-cancer effects of Cu are discussed in depth.

Search Approach and Presence Criteria

This review focused on the 'pharmacotherapy and management of cancer with Cu. The keywords of Cu, cucumber anticancer therapy, cytotoxic effects, chemotherapy, and inhibitor effect were searched until February 2020. The list of articles on International Pharmaceutical Abstracts, PubMed, EMBASE, OVID, Scopus, and Google Scholar were investigated.

The main word searched were Cu, anticancer activity, chemotherapy, cytotoxic effects, Cu E, together with 'medicines' and 'drugs'. The word 'cuc' was investigated in combination with inhibitors, cancer, and 'apoptosis, cell cycle, cucurbitaceae family. The search was done from September 2018. The final studied article that mention in references was 99 articles.

Biological Activities of Cucurbitacins

Anti-inflammatory activities

Cu R, Cu D and Dihydro cucurbitacin B (DHCB) showed anti-inflammatory affect.[15-20] 23, 24-dihydrocucurbitacin B and Cu R showed carrageenan-induced mouse paw edema, serotonin-induced mouse paw edema, phospholipase A2-induced mouse paw edema, 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced acute ear edema in rat polymorpho nuclear leukocytes. They are also inhibited inflammation, caused by continual treatment of TPA in mouse, then effect on edema as well as cell infiltration.[16,19] Inflammatory tissues exhibited that DHCB reduces the cytokines implicated in these processes, including interleukin-1 beta, interleukin-4, tumor necrosis factor-alpha and also the interleukins production in human T lymphocytes, inhibits the proliferation of phytohemagglutinin-stimulated human T lymphocytes, cell cycle in the G(0) phase, including A(1), B(1), D(2), and E(1), nuclear factor of activated T cells (NFAT), and the induction of the principal cyclins in human lymphocytes without affecting the influx of calcium[17] Finally, these findings recommend that dihydro cucurbitacin B by inhibiting NFAT limits Delayed-type Hypersensitivity (DTH) reactions, which prevents the proliferation of cells complicated in DTH reactions, like T cells.

In addition, intra-peritoneal treatment of mice with DHCB, showed the production of Prostaglandin E2 (PGE2), by Cyclooxygenase-2 (COX-2) transfected COS-7 cells that was noticeably suppressed.[18] Additionally, Cu B (CuB) can efficiently advance function of pulmonary gas exchange, reduction of pulmonary edema, and reduction of inflammatory reaction in lung.[20]

In macrophages, CuD has ability to enhance the production of LPS-induced interleukin (IL)-1β in culture media of THP-1 cells, peritoneal exudate cells (PECs), and RAW264 cells bone marrow derived macrophages (BMDMs). Transcriptional level analysis showed that CuD increased expression of LPS-induced IL-1β mRNA via ERK1/2 mitogen-activated protein kinases (MAPKs) activation. At the post-transcriptional level, CuD induced the activation of caspase-1 subsequent treatment with a caspase-1 inhibitor and siRNA. Further, CuD has been exhibited to induce activation of inflammasome independent of ERK1/2 activation. Results of Western blotting revealed interactions between pyrin domain containing 3 (NALP3), NOD-like receptor family and apoptosis-associated speck-like protein that suggested the inflammasome activation and probably led to caspase-1activation. In total, it can be resulted that CuD has ability to induce immunomodulating activity in macrophages which led to inflammasome activation in addition to improvement of LPS signaling.[21]

Hepatoprotective effects

CuB, CuE, and their mixture, reduce concentration of serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), TP, ALP, and TBIL which in turn decrease degradation of necrosis of hepatocytes and thereby rats protection from injury of CCl4-induced acute liver. CuB has also showed protection against fatty liver, hepatic cirrhosis and hepatic fibrosis thereby inhibiting apoptosis in primary cultured neonatal rat hepatocyte.[22]

Antiviral activities

Cucurbitacins have been treated in chronic hepatic carcinoma and viral hepatitis, but the mechanism of action in not clear.[23-24] The Epstein–Barr is a key agent in nasopharyngeal carcinoma. 23, 24-dihydrocucurbitacin F, Scandenoside R6, 24-dihydrocucurbitacin F, 25-acetyl-23, 2-O-beta-D-glucopyranosyl-23, and CuF have been known as inhibitors agent of Epstein–Barr virus antigen activation.[25-26] In an in-vivo carcinogenesis of mice skin papillomas Cu have delayed the papillomas formation and decreased the tumor number.[26] Recently, has reported, the anti-HIV-1 activity of cucurbitacin B in C8166 cells (EC = 0.09mg/ml).[27] CuB has synergistic antiviral and antibacterial properties against *Herpes simplex virus-1* and *Staphylococcus aureus*.[28] CuB at different dose in combination with other antibiotics such as oxacillin and tetracycline inhibited *Staphylococcus aureus* growths which are clinical drug resistant variant. Also clinical assay showed that it has potent anti-HSV-1, in comparison to acyclovir. CuB has potential effect on decrease in the behavior and sexual hormone of *Agrotis ipsilon*.[29] CuB inhibited central pheromone processing in a dose-dependent manner. In a study synergistic effect of The PHA/CuE combination on ZR-75-1 cell line was investigated. Results showed that the activation of cytotoxic T-cell subset effectively resulted in ZR-75-1 cell death. This result is consisting with another experiment[30] in which activated lymphocytes released IL-6[31] and also an interleukin that has considerable activated against ZR-75-1 cells *in vitro*. Previous study indicated potential effect of CuE on PC-3 cells in comparison to the ZR-75-1 cells

Antifertility

Women obtained ancient information such as used botanical herbs for the control of pregnancy. In this purpose, researchers find a strong antifertility agent with least side effects from plants that can be developed as a synthetic medicine.

Some Cu showed to have antifertility activity. Enriched Cu extract has suppressed the number of occurrences of the estrus stage and uterine implantation. Plant derivative has potential inhibitory effect on sperm membrane specific enzyme such as hyaluronidase and acrocin that are significant enzyme involved in the fertilization process. Cucurbitacin has potential effect on sperm motility and viability. In the fertility process, sperm motility in cauda-epididymis is important. Immobility and concentration of spermatazoa has important role in the penetration in to cervical mucus and then fertilization of ova.[32]

Anticancer effects

Cu has shown proliferation suppression in different types of cancer cell lines.[33] Cu make numerous physiological and morphological changes in cell shape, multi nucleation and cell cycle arrest in cancer cell lines.[5] The efficiency of Cu D, B, E, and I, have shown in colon, lung, breast and brain cancer cells. Similarly, Cu A, B, E, I and Q exert anti-proliferative effect on lung cancer cells. CuB, I, E, C, D, and K promote TRAIL-induced apoptosis with sensitizing renal adenocarcinoma cells to anticancer property of TRAIL in addition to synergistic consequence following short contact with no need of exposure.[3]

CuB has intense anti-proliferative property on human pancreatic cancer cells, K562 cells, various leukemia and lymphoma cell lines, GBM cells , lung cancer (A549), colon cancer (Caco-2) and breast cancer cell lines (T47D, MCF7, SKBR3, and MDA-MB435).[34-39] CuD and I showed cytotoxicity effect on BEL-7402 and A549/ATCC cells.[39] Cucurbitacin D exhibited potent anti-cancer activity in cervical cancer.[40] CuI showed growth reduction in prostate and breast carcinoma cells, in vitro, as well as in nude mice xenograft model. Besides CuI demonstrated anti-cancer property in B leukemic cells.[41] CuE and I glycoside have strong in vitro cytotoxic activity against Hepatoma cells and extended survival phase, life period and normalize the biological factors of mice-bearing tumor in Ehrlich's ascites carcinoma.[3]

Anticancer mechanisms of cucurbitacins: Signal transducers and activators of transcription

In the production of cancer cells, and their survival, a large number of oncogenic signaling pathways are involved.[42,5] Most remarkable mechanism of Cu is their capability to change transcriptional levels through genes or nuclear factors by which they can inhibit or activate anti/apoptotic proteins. They select as an inhibitors of some action such as JAK/STAT pathways, MAPK pathways, PARP cleavage, activate caspase-3 expression, decreased JAK3 and pSTAT3 levels, in addition to decreases in several downstream STAT3 targets such as Bcl-xL, Bcl-2, Mcl-1, and cyclin D3, that they are involved in cell cycle and apoptosis.[43,44] For instance, CuB affects the Raf/MEK/ERK pathway in the K562 cells and the MAPK pathway in glioblastoma multiform (GBM) cells.[5,35] Additionally, CuD promotes apoptosis via caspase-3 and JNK phosphorylation in hepatocellular carcinoma cells.[40] On the other hand, the anticancer activity of Cu can be related to their anti-inflammatory activity because cancer can be induce and/or intensified by infections and inflammation.[5,45] In cancer cell lines, Cu inhibited STAT3, that led to more cell susceptibility in the inhibition of reactive oxygen species (ROS) and also free radicals during inflammation. In macrophages, Cu have inhibitory effect on IKK/NF-κB pathway which led to the inhibition of main inflammatory enzymes, like cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS), whose over productions involved in tumor genesis.[5] In addition, the side chain has a relevant role in several aspects of their pharmacological activity. The molecules with a double bond exhibit increased cytotoxicity while also inhibiting cell adhesion properties as compared to those without the double bond.[4] Tumor cells obtain the capability to increase uncontrollable proliferation, resistance to apoptosis, sustainable to angiogenesis and then avoid immune surveillance. STAT proteins specifically STAT3 and STAT5 normalize all of these procedures and are insistently activated in an unexpectedly large number of human cancers.[46] STATs have been revealed to have vigorous roles at tumorgenesis. They have responsibility in the generating proliferative signals and have been revealed to up-regulate anti-apoptotic proteins. Furthermore, STAT3 confirmed to up-regulate the VEGF expression that is essential for angiogenesis and the conservation of tumor vasculature. STAT3 has been involved in the inhibition of immune responses of tumor growth through stopping the pro-inflammatory factors expression. Stimulation of STAT3 and STAT5 unregularly confirmed in a wide range of tumors, including prostate, breast, multiple myeloma, leukemia, and melanoma.[47] Activation of the STAT3 meditated signaling pathway is a main factor to oncogenesis, cell growth and survival (Figure 1).[3,42] Convincing proof of mechanistic researches with peptides, antisense, RNA interference (RNAi), and small molecular inhibition showed that stopping STAT3 signaling make effective inhibition of tumor cell growth and apoptosis.[48] In lung cancer A549 cell line, CuA, B, E, I and Q avoid STAT3 DNA binding and STAT3-mediated gene transcription.[49,50] Similarly, CuI make phospho-STAT3 reduction in breast prostate and pancreatic carcinoma cell lines (MDA-MB-468, MDA-MB-231, and Panc-1). CuB and E demonstrated to make STAT3 phosphorylation in breast cancer cell lines (MCF-7 and MDA-MB-231).[51] CuI, B and Q prevent STAT3 phosphorylation and also apoptosis induction in v-Src-transformed NIH3T3 cells.[40] In addition, CuI has inhibitor effect on JAK-STAT in different human cancers. Furthermore, CuB prevents the tyrosine phosphorylation of STAT3, STAT5 and JAK2 in pancreatic

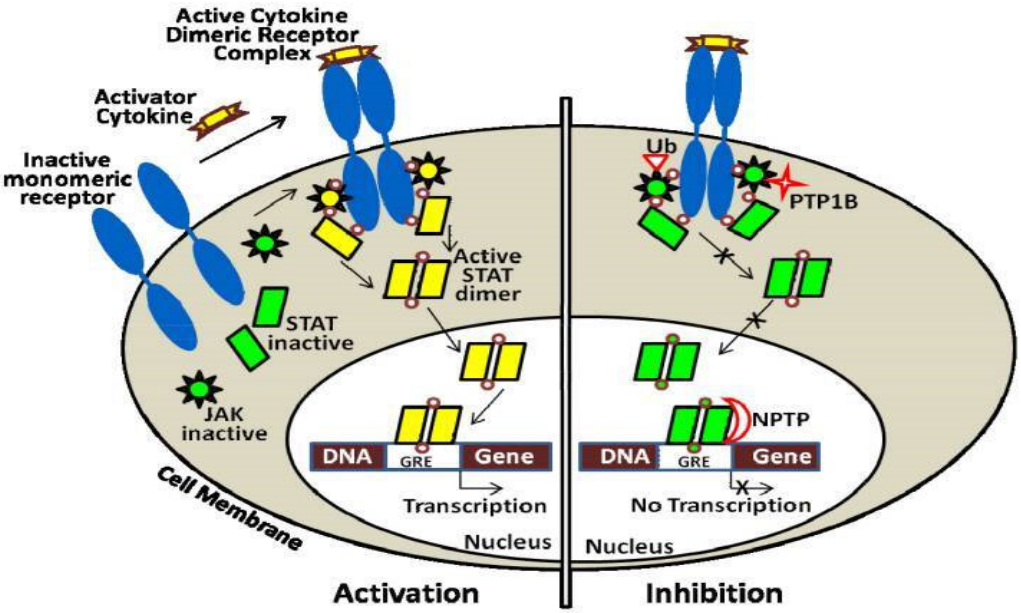

Figure 1. JAK/STAT pathway: Upon activator cytokine binding to its receptor on cell surface, JAK/STAT pathway is activated (left) leading to sequential cell response. Inhibition of signaling process (right) is induced by an inhibitory cytokine, JAK degradation through ubiquitin-proteasome system (Ub), dephosphorylation by cytoplasmic PTP1B or nuclear phosphatase (NPTP), or by inhibition the dimerization of STAT.[3]

cancer cell lines (MiaPaCa-2 and Panc-1) *in vitro* and in Panc-1 xenografts in vivo. CuB/E glycosides increase the tyrosine phosphorylation of STAT3 in breast cancer cell lines (MCF-7 and MDA-MB-231).[5] CuI quickly inhibit phosphor tyrosine STAT3 in human lung adenocarcinoma A549 cancer cells, and v-Src-transformed NIH-3T3 cells while CuQ selectively inhibits STAT3 in A549 cells.[22]

Cell proliferation

CuB has anti-proliferation effects on different types of tumors. CuB inhibits Hep-2 cells proliferation and B16F10 a mouse melanoma cell line.[52,53] The half-maximal inhibitory concentration (IC_{50}) value for CuD and I on A549/ATCC and BEL-7402 cell lines is less than 1 μM.[38] In addition, CuE and B have shown the better cytotoxic properties and their IC_{50} values are equivalent to camptothecin.[54] Table 1 showed the antiproliferative effects of CuB, D, and E on cancer line cells.

Apoptosis and cell cycle arrest

Following treatment with Cu, the most common manner of cell death is apoptosis. Two key apoptotic ways occur:

Table 1. The antiproliferative effect of cucurbitacins B, D, and E on cancer line cells

	Cucurbitacins B	Cucurbitacins D	Cucurbitacins E
MDA-MB-231	3.03×10^{-8} (mol/l)/48 h/4×10^3 cells[a,55]	-	-
BEL-7402	0.32 (mmol/l)/48 h/7×10^3 cells[56]	< 1 (mmol/l)/72 h[b]	42.9 (nmol/l)/72 h/5×10^3 cells[57]
Panc-1	10^{-7} (mol/l)/24 h/10^4 cells	-	37.03±2.22 (mmol/l)/24 h/5×10^3 cells 3.05±1.18 (mmol/l)/48 h/ 5×10^3 cells 1.10 ±0.53 (mmol/l)/72 h/5×10^3 cells[58]
U937	2.5×10^{-8} (mol/l)/96 h/ 10^4 cells[59]	2.0×10^{-7} (mol/l)/96 h/10^4 cells	-
Hep-2	115±12 (mmol/l)/24 h/5×10^3 cells, 34±3 (mmol/l)/48h/5×10^3 cells, 3.9±0.6 (mmol/l)/72 h/5×10^3 cells[59]		-
PC3	-	-	10 (nmol/l)/48 h[b,60]
A549	41.13±0.19 mg/ml/48 h/ 1×10^4 cells[61]	<1 (mmol/l)/72 h[b,62]	-

3.03×10^{-8} (mol/l)/48 h/4×10^3 cells in the table means for example the IC_{50} for cucurbitacin B in MDA-MB-231 cell is 3.03×10^{-8} mol/l, which is achieved by treating 4×10^3 cells with cucurbitacin B in different concentrations for 48 h. [b]The treated cell number was not specified in the reference

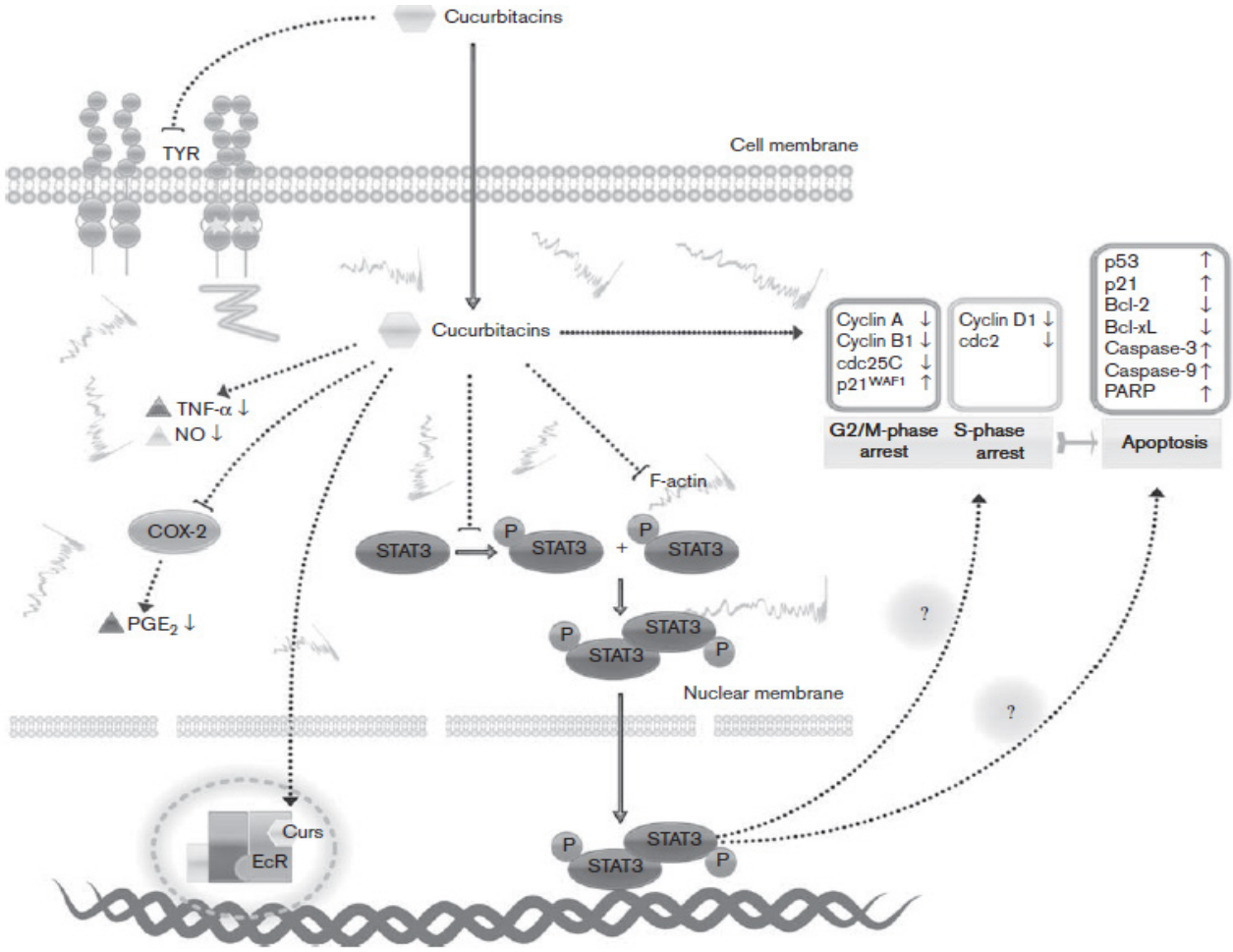

Figure 2. Molecular targets for cucurbitacins: cucurbitacins inhibit COX-2, TYR, and the phosphorylation of STATs, mainly STAT3. Cucurbitacins are identified as insect steroid hormone antagonists acting on the EcR as well. Finally, different cucurbitacins could induce G2/M and/or S-phase cell cycle arrest, which might lead to apoptosis.[22] Copyright (2012) Wolters Kluwer Health, Inc., Licensee Number: 4880751034555.

death receptor mediated pathway (extrinsic pathway) and mitochondrial pathway (intrinsic pathway). Arrest of Cu induced cell cycle is often complemented by apoptosis as well (Figure 2).[22] Various types of Cu can make various phases of cell cycle arrest.[63] CuD can suppress the proliferation and promotes the apoptosis of T-cell leukemia cells by down-regulation of anti-apoptotic proteins like Bcl-xL and Bcl-2 expression.[64] CuB and their derivations treatment makes induction of S phase stopping and apoptosis in hepatocellular carcinoma. The growth suppression effect is related to down regulations of cyclin D1 and cdc-2.[65] CuB treatment in HepG2 cells led to cells accumulation at the S phase in cell cycle along with apoptosis by inhibiting down-regulation of Bcl-2 expression.[66] CuB inhibited growth of PANC-1 cells (Pancreatic cancer) by G2/M phase cells accumulation and apoptosis. Moreover, CuB treatment down-regulate the Bcl-2 expression and survivin. CuB induces G2 phase arrest and apoptosis, expression reduction of cyclin B1 and cdc25C proteins, also activates caspases in a dose-dependent way in human colon cancer SW480 cells.[67] CuB shows major efficacy in the inhibition of growth, cell cycle inhibition at G2/M phase, and apoptosis induction due to inhibition in p-STAT3, Bcl-2, and cyclin B1 expression. Also, tumor growth can inhibits by CuB in a dose-dependent way in Hep-2 cells and in vivo researches in a mouse xenograft model.[68] 23, 24-Dihydrocucurbitacin B, found to inhibit the proliferation of HeLa, Bcap37, SMMC-7721, K562 SW620 and MCF-7, human cancer cell lines and induce apoptosis in Bcap37 human breast cancer cell line. DHCB induces Bcap37 apoptosis through mitochondrial dependent pathway and stopping induction of G2/M phase in cell-cycle as well.[69] Treatment of Myeloid leukemia cells with CuB showed stopping of S-phase in cell cycle, enlarged cell size, multi-nucleation, and increase in a monocytic- and granulocytic-specific CD11b expression.[35] In addition, CuE induces apoptosis in MDA-MB-468, SW527, MDA-MB-231 and Bcap37 cells and also decreases the anti-apoptotic proteins levels such as Mcl-1, XIAP, and Survivin.[45]

Cell differentiation

Cu can encourage cancer cell division. For example CuI

restore tumor consequential factor that induced separation suppression in macrophages and dendritic cells (DCs), reduces the occurrence of immature myeloid cells and enhanced the buildup of grown-up DCs.[70] The treatment of U937 cells and HL-60 with CuB induces granulocyte particular CD11 expression.[36] CuI antagonizes suppression effect of interleukin (IL)-6 on CCAAT inducer binding protein in CD33+myeloma cells (the key transcription factors that mediate granulocyte differentiation) gene expression.[71] Furthermore, CuI induce the differentiation of CD133+ to CD133– in non-small cell lung carcinoma and CD44+ALDH1+ cancer cells to CD44 –ALDH1– Cells isolated from neck and head squamous cell carcinoma patients.[72] In a study CuE inhibits cellular proliferation and enhances the chemo-response in gastric cancer by suppressing AKt activation.[73]

Invasion and metastasis

Cus have shown anti-invasion activities. CuI can reduce the invasiveness of invasive nasopharyngeal carcinoma with elevated the activation of STAT3.[74] CuB can potentially inhibit 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced cell invasion and migration in a concentration-dependent way, which is along with with inhibition of TPA-induced MMP-9 expression via phosphorylation inactivation of extracellular signal-regulated kinase (ERK) 1/2, p38 and Akt in human hepatoma HepG2 and BEL-7402 cell lines.[75] In neck and head squamous cell carcinoma, CuI suppresses invasion, colony formation, and tumor sphere formation. Additionally, CuI suppress distant tumor metastases and decrease the volume and number of tumors in tumor-bearing mice. Furthermore, in the colon cancer cell line HCT8/S11, several Cus are reported to dose-dependently inhibit vertebrate trefoil factors 1 and 3 in addition to VEGF-induced invasion. CuI have revealed analogous effects on NPC, HK1, and CNE-2 cells, in addition to in an endometrial cancer Matrigel model. CuB inhibits the squamous cell carcinomas SRB1 and SRB12 migration as well.[21]

Fibrous-actin

Cu showed strong effect on cell cytoskeleton (human stellate cell line (LX-2)) by largely effect on actin by induction of both aggregation and depolymerization.[76] CuE interacts with actin therefore stabilizing polymerization of actin. When CuE is treated with NIH-3T3 cells exogenously expressing YFP-labeled actin, firstly globular actin aggregation and then actin aggregation together with interrupted fibrous actin in cells was observed.[76] CuI treated cells disclose disassembly of F-actin fibres, reformation into F-actin pieces and declaration of grip.[77] CuI potently decrease mobility and aggregation in Madin-Darby canine kidney (MDCK) cell sheets and B16-F1 mouse melanoma cells. When CuI treated with MDCK or B16-F1 cells, there is extremely speedy termination of motility and regular buildup of filamentous actin aggregates. In actin depolymerization there are two proteins that are actin-severing proteins gelsolin and cofilin. CuI results in buildup of actin filaments in cells by exclusive indirect mechanism.[78] CuIIa encourage irreversible bunch of filamentous actin and prevented cell cycle by increase in G2/M populations. CuIIa make decrease phospho-Histone H3 and increases cleavage of poly-(ADP-ribose) polymerase or PARP, upstream of DNA breakdown, steadily with mitotic blockage-induced cell death. CuIIa guides the cell for PARP-mediated apoptosis via suppression of survivin downstream of JAK2/STAT3.[42] In addition, the G-actin groups were used up and then actin aggregation were created rapidly after CuB treatment in murine B16F10 melanoma cells. Also CuB makes rapid reduction of the G-actin groups via aggregation of ROS-dependent actin in melanoma cells, which may finally use for its anti-tumor activity.[79] Moreover; CuB obviously changes the cytoskeletal complex of leukemic cells, inducing fast and unsuitable polymerization of the F-actin.[27]

Tyrosinase

Tyrosinase is a key enzyme in melanin biosynthesis. Although melanin has important role in human cell protection, but melanin hyper-pigmentation result in serious aesthetic problems. Thus tyrosinase inhibition is a potential application in medical. 23, 24-dihydro-cucurbitacin D and CuD was extracted from *Trichosanthes Kirilowii* by tyrosinase inhibitory activity-guided fraction. Both compound showed effective inhibition of tyrosinase activity, and also the synthesis of melanin in B16/F10 melanoma cells.[80]

Synergistic effects

In cancer treatment, a common practice is multiple drugs usage. The combination antitumor effect of docetaxel with CuB on Hep-2 (a human laryngeal cancer cell line), showed more ability in growth suppression, cell cycle inhibition at G2/M phase as well as apoptosis initiation by suppressing of Bcl-2, cyclin B1 and p-STAT3 expression, compared to individual therapy. Additionally, CuB with docetaxel showed synergistic trend for inhibition of tumor growth in a mouse xenograft model.[81] In chemotherapy, interaction between doxorubicin (DOX) and CuB and C enhanced effect of anticancer drug.[82] The grouping of CuE with DOX results in reduction of tumor weight and tumor size, in comparison to DOX alone.[83] Moreover, CuE can improve the DOX level in M5076 ovarian sarcoma through inhibited DOX efflux in vitro.[84] Co-organization of DOX and CuD prompts G2/M cell cycle arrest and apoptosis as well as inhibition of sup-regulated STAT3. Additionally, CuD directs enhancement of IκBα level in the cytosol and reduction of p-NF-κB level in nucleus. It was demonstrated that CuD slows down translocation of NF-κB and STAT3 then reduces transcriptional activity in nucleus. Alternatively, CuD reduces cell abundance and stimulates apoptosis by restraining NF-κB and STAT3 signaling in MCF7/ADR breast cancer cells resistance to doxorubicin.[63] Co-treatment with ethanol notably enhances cytotoxicity

of CuB. Ethanol can reduce CuB-induced mitochondria membrane potential (ΔΨ) depolarization. In addition, ethanol can improved apoptosis induced by CuB. Ethanol inhibits CuB-induced autophagy and autophagy protein expression. Consequently, ethanol improves cytotoxicity of CuB in LO2 hepatocytes, which is intermediated by restraining autophagy and enhancing apoptosis.[85] B16F10, is a mouse melanoma cell line that is more sensitive to the combination of CuB with chloroquine. When CuB is joined with VPA (vasoactive intestinal peptide termed VPAC), the both drugs show synergistic cytotoxicity effect by induction of cell apoptosis.[42]

Cucurbitacin E and Biological Activities

Structure

Basic skeleton of CuE has 30 carbon atoms and so its lanostane skeleton is multi-substituted with methyl, hydroxy and oxo substituents, with unsaturation at positions of 1, 5 and 23 that is having variety of biological activities, together with antitumor effect, anti-chemical carcinogenesis, liver protection, improvement of immunity, etc.[40, 86, 87] Conversely, CuE shows most anticancer outcome amongst cucurbitacins in a variety of cancers[88-90] (Figure 3).

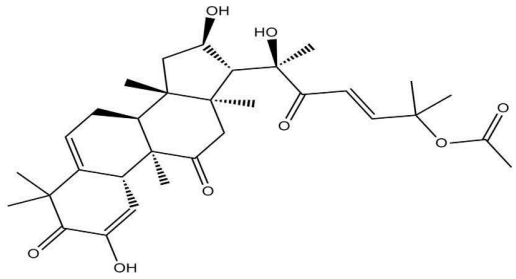

Figure 3. Chemical structure of cucurbitacin E

Breast cancer

CuE showed noticeable inhibition of mouse and human breast cancer metastasis, 4T1 MDA-MB231 and cell migration, also invasion in low concentration. CuE reduces Arp3 (a subunit of the Arp2/3 complex which engages in actin polymerization) and actin expression. In addition, CuE established to suppress the activity of FAK and c-Src phosphorylation and reduce expression of FAK/c-Src downstream proteins together with, Rac1, JNK and c-Jun which considered as the main mechanisms of cell migration and invasion. Up regulation of Matrix metalloproteinases (MMPs) and MMP9 can improve metastasis of tumor cell probably in breast cancer and linked to invasive performance of tumors. Alternatively, Tissue inhibitor of metalloproteinase 1 (TIMP 1) and TIMP-2 suppress these activities and are identified for their inhibitory outcome on tumor metastasis. CuE not only down-regulates the MMP2, MMP9 expression, and reduces their activity but also up-regulates TIMP1 and TIMP2 expression in cell lysates and primary tumors.[90]

Triple negative breast cancer (TNBC)

The resistance of Triple Negative Breast Cancer (TNBC) has encouraged huge research. CuE change anti apoptotic proteins Survivin, cell cycle protein Cyclin D1, XIAP, Mcl-1, Bcl-2, and numerous signaling pathways like pERK, pJNK, pSTAT3, pAKT in mainly susceptible TNBC cell lines. It encourages G2/M cell cycle arrest, apoptosis in SW527 TNBC and MDA-MB-468 cell lines by decreasing levels of Survivin, Bcl2, XIAP, and Mcl-1. In addition, CuE considerably decreases levels of pAKT, pERK, and total AKT and noticeably improves levels of p-c-Jun and pJNK in MDA-MB-468 cells.[40]

Bladder cancer

CuE induce G2/M phase arrest in Bladder cancer T24 cell lines by reducing levels of phospho-STAT3, CDK1 and CDK2 kinases protein and cyclin B while increasing levels of p53 and p21 (Figure 4).[2]

Brain tumor

Triterpenoids, which are steroidal compounds, are capable of crossing BBB because of their lipophilic nature therefore they can be accountable for exerting antitumor activity in brain.[8,91]

CRC (Colorectal cancer)

CuE arrests the growth of primary CRC cddis 2014151 cell lines by inducing of mitosis in GBM8401 cells by interrupting the G2/M phase cell cycle through GADD45g gene expression and hindrance of cyclin B1/CDC2. Particularly, GADD45g is competent to hold back G2–M succession in reaction to stress in the course of its capacity to act together with, and hold back the kinase activities of the cyclin B1/CDC complex. So, RNA silencing of GADD45 expression damages activity of G2–M checkpoint. Furthermore, down-regulation of GADD45 is intimately linked to the degree of malignancy in cancers. In addition, it reduces the tumor growth and improves VEGFR2-mediated Jak2–STAT3 pathways result in apoptosis and anti-angiogenesis.[8]

Prostate cancer

Both primary prostate carcinoma explants and immortalized cells of prostate carcinoma seem particularly sensitive to suppression by CuE.[92] CuE led to generation of morphologically abnormal, multi nucleated cells and a cytokinetic block. CuE causes clear disturbance of the actin cytoskeleton complex and in a sequence of Cu analogues, anti-proliferative action relates straight with disturbance of F-actin cytoskeleton. Interruption of actin cytoskeleton links with anti-proliferative effect in a sequence of Cu congeners. Dissemination of vimentin is also changed in cells, as vimentin links with drug induced membrane blebs.[92] CuE prompts autophagy via mTOR and AMPK signaling pathway in LNCaP cells. The mTOR signaling pathway over activation enables growth promotion and proliferation of cancerous cells while inhibiting apoptosis and autophagy as well as promotes cell migration, metastasis, invasion, and angiogenesis. Moreover, AMPK controls development of apoptosome via caspase-9 precursors following of

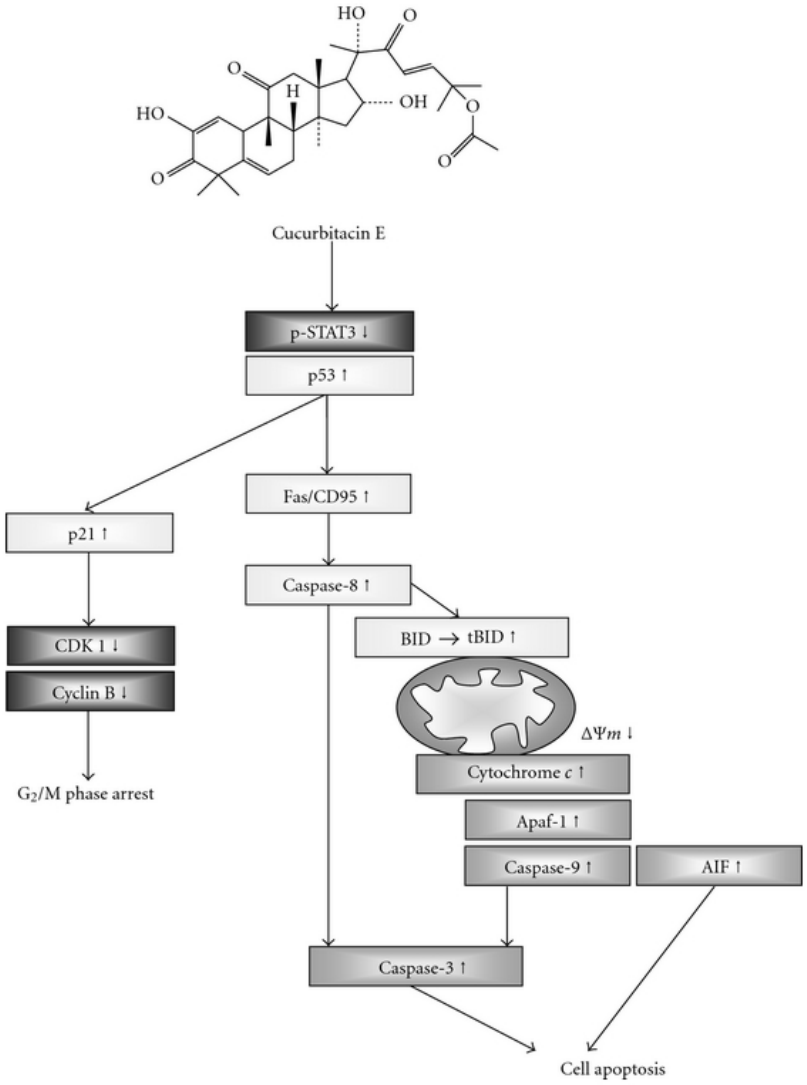

Figure 4. Effects of cucurbitacin E in human bladder cancer: STAT3/p53/p21 signaling, apoptosis, and mitochondria-dependent pathways.[2]

activation of p53 signaling pathway. AMPK triggers some of proteases, endonucleases and suppress DNA repair, which result in destruction of nucleoproteins cytoskeletal proteins that results in apoptosis.[89]

Oral squamous cell carcinoma

CuE causes a notable alteration in morphology of Human Oral Squamous Cell Carcinoma (SAS) and encourages death of cancer cells by increasing apoptosis and cell cycle arrest. Results of experiment provide evidence that demonstrated CuE irreversibly inhibited growth of tumor cell. From mechanistic analysis, it can be concluded that CuE accumulation is a key factor in the proliferation inhibition and the cell cycle arrest induction in the cancer cells. Apoptosis induction by CuE is mediated through the caspase 3 activation. Caspases initiate the apoptotic program. Caspases have main role in the regulation of apoptotic cell death. Firstly, all produced caspases are inactive zymogens in cells, that after a proteolytic cleavage they convert into active form during apoptosis. Cleaving caspases, initiates the induction of apoptosis in tumor cell.[93]

Ovarian sarcoma

CuE causes morphological alterations and apoptosis in ovarian sarcoma ES-2 cell lines. As the concentration of CuE increases, the number of ES-2 cells significantly decrease or the time extended. Ratio of ES-2 cells escalates both in S phase and in G2/M phase, while reduces in G1 phase. Furthermore, CuE hinders STAT3 phosphorylation in ES-2 cell in a dose dependent way.[68,64] Besides, CuE raises DOX level through destruction of DOX efflux in M5076 ovarian sarcoma cells. MRP5 overexpression is definite in M5076 ovarian sarcoma cell membranes as well. MK-571, as an inhibitor of Multidrug resistance protein (MRP) considerably defeats DOX efflux in M5076 ovarian sarcoma cells. Combination of CuE and MK-571 hinders

DOX efflux as well. DOX concentration is augmented in tumors and reduced in normal tissues subsequent to CuE co-treatment. This dissimilar effect is a result of alterations in MRP sub types.[84]

Pancreatic cancer (PC)

CuE treatment made cells cycle arrest at G2/M phase via caspase 8 and 9 activation. Treatment with CuE induced apoptosis in human PC cells via cofilin 1 and mTORC1 signaling. These results suggested that CuE may be a potential therapeutic candidate for the treatment of PC.

CuE prevents STAT3 phosphorylation while up-regulates p53 expression in human PANC-1 cells. The p53 protein capture cell cycle development at numerous check points then encourage apoptosis in unrestricted growth cells. The p53 inhibition is facilitated by activated STAT3, which binds to the p53 promoter both in vitro and in vivo. Moreover, activation of STAT3 has the ability of hinder endogenous p53's for the controlling of reactive genes.[58]

Pharynx and nasopharyngeal carcinoma

CuE has the ability of growth restriction in Detroit 562 (human pharynx carcinoma) and HONE-1 (human nasopharyngeal carcinoma) cancer cells by interruption of mitosis by down-regulation of phosphorylation and expression of cyclinB1 and CDC2 protein. Furthermore, CDC2 proteins can be linked with GADD45c that assists in G1 and G2/M phases in cell cycle, which are significant for anti-tumor immune reactions.[13]

Other cell lines

CuE presented strong cytotoxic properties in human leukemia (U937) cells.[94] Furthermore, CuE has prospective as an anti-angiogenie agent in tumor therapy.[95] CuE make cell growth capture and apoptosis by promotion of Eukaryotic Initiation Factor 2 (eIF2) phosphorylation, that result in inhibition of Mcl-1, survivin, cyclin-dependent kinase 1, and/or X-linked inhibitor of apoptosis protein (XIAP) protein synthesis. It creates apoptosis primarily by mitochondrial pathway in human leukemia HL-60 cells.[96] CuI and E hinder phosphorylation of cofilin in concentration-dependent way in HT1080 cells, and their effective concentrations of them showed similar cytotoxic effect in U937 cells. Furthermore, after CuE treatments the fibrous-/globular-actin ratio have decreased.[94]

Lymphocyte modulation

Cytotoxic effects of some plant extracts were studied on malignant cells, should also be tested for their probable stimulatory properties on the activation of lymphocyte. This is particularly considered for in vivo studies of drugs.[97] CuE was tested for their immune modulatory effect on peripheral human lymphocytes. Co-culture of peripheral human lymphocytes with cancer cells showed a remarkable lymphocyte-mediated cytotoxicity.[98] CuE supports proliferation of activated lymphocytes, deprived of antagonist effects on their morphology and it has a stimulatory effect on normal lymphocytes. Activation of T-lymphocyte by mitogens is through binding to T-cell receptors, with several CD3 molecules that spread signals to inside the cell. Transfer of signaling through CD3-TCR pathway includes expression of transcription factors c-myc, c-fos, and c-jun. Subsequent to that, lymphocytes undergo variations leading to an enhance in intracellular Ca^{2+} levels and initiation of protein kinase C. Likewise alterations seem to be complicated in facilitating T-cell apoptosis. Certainly, same signals are essential for proliferation of T cells seem to be essential for growth inhibition and apoptosis origination in T cells activation. CuE acts by CD3/TCR pathway as prelude to activation of lymphocyte and ensuing apoptosis. It seems that a drug with cytotoxic effect on cancer cells line have an enhancing effect on immune system, which is vital in cancer.[98]

Conclusion

Different studies have obviously demonstrated that Cus are an important class of active triterpenoids in plants. Here, review of their biological activity during 10 years has probably showed the significance effect of natural bio-products in drug detection. Structures of Cu are described by the skeleton of tetracyclic Cu nucleus (triterpenes), because of their hydrophobic properties and poor solubility in water, and polymeric micellar systems showed enhanced antitumor activity due to well solubilization and targeting after local and systemic organization. Various Cu compounds showed inhibition of antitumor proliferation and induction of apoptosis alone or with other established anticancer chemicals complexes and cytokines in several human cancer cell lines and tumor xenografts of leukemia, breast, prostate, lung, uterine cervix, lymphoma, skin, colon, liver, brain, laryngeal, and pancreatic cancers. In a structure-function related way, Cu can inhibit the phosphorylation of STAT3 and/or JAK2 and later activation appeared as the major mechanism of their action.[13] In conclusion, future studies targeting their researches in various sources that are uninvestigated and also derivatives of Cu for promoting their anticancer activities in addition to other activities in in vivo condition.

Conflict of Interests

The authors claim that there is no conflict of interest.

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