DOI: 10.55415/deep-2022-0032.v1

Brusatol, a traditional medicine compound, can modulate diverse oncogenic targets for cancer therapeutics

Running title: Brusatol can modulate diverse oncogenic targets

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DOI: 10.55415/deep-2022-0032.v1

ABSTRACT

Cancer is an unexpected consequence of uncontrolled cell proliferation that is related to cell cycle disorganization, and a multifactor-caused disease that needs the modulation of numerous oncogenic signaling pathways and targets. The battle with this malignance has been waged for centuries whereas this dreadful disease is still an important cause of death all over the world. Due to the development of resistance to current anticancer drugs, lots of efforts are made to discover more effective agents for tumor therapy. Meanwhile, natural products possess a powerful prospect for anticancer drugs discoveries. Brusatol, a component isolated from natural plant Brucea javanica, was able to efficiently combat with wide variety of tumors. Extensive researches had shown that Brusatol exhibited its anticancer capability by arresting cell cycle, promoting apoptosis, inducing autophagy, attenuating epithelial-mesenchymal transition, inhibiting migration, invasion and angiogenesis as well as raising chemosensitivity and radiosensitivity. In addition, these involved various oncogenic signaling pathways, including MAPK, NF-κB, PI3K/AKT/mTOR, JAK/STAT and Keap1/Nrf2/ARE signaling pathway. The current review describes in detail and discusses the various reports supporting the possibility of Brusatol to be a hopeful drug candidate for cancer therapeutics.

Keywords: Brusatol, cancer therapy, molecular target, Traditional Chinese Medicine (TCM)

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ABBREVIATIOOS: HNSCC, head and neck squamous cell carcinoma; MM, multiple myeloma; NPC, nasopharyngeal carcinoma; TCM, traditional Chinese medicine; CPT, Camptothecin; EMT, epithelial-mesenchymal transition; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-B; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; JAK, janus tyrosine kinase; STAT, signal transducers and activators of transcription; Nrf2, nuclear factor erythroid-2 related factor 2; WHO, World Health Organization; CDKs, cyclin dependent kinases; CKI, cyclin dependent kinase inhibitor; MMP, mitochondrial membrane permeability; Bcl-2, B-cell lymphoma-2 proten; Bax, Bcl-2-associated X proten; Bak, Bcl-2 homologous antagonist/killer; Cyt C, Cytochrome C; Apaf1, apoptosis protease activating factor 1; Fas-L, Fas ligand; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptors; TRAIL, TNF-related apoptosis-inducing ligand; ATG, autophagy-related protein; ULK, Unc-51-like autophagy-activating kinase; LC3 II, microtubule-associated protein 1 light chain 3 II; HCC, hepatocellular carcinoma; MMP-9, matrix metalloproteinase-9; EGF, epidermal growth factor; MMPs, matrix metalloproteinases; FGF2, fibroblast growth factor-2; VEGF, vascular endothelial growth factor; ROS, reactive oxygen species; ATR, ataxia telangiectasia and rad3 related protein; CHK1, checkpoint kinase 1; CDDP, Cisplatin; TUNEL, TdT-mediated dUTP nick-end labeling; ERK1/2, extracellular regulated protein kinases 1/2; HO-1, heme oxygenase-1; CSCs, cancer stem cells; HIF-1α, hypoxia inducible factor-1α; PHDs, prolyl hydroxylases; AKR1C1, aldoketo reductase family 1 member C1; ARE, anti-oxidative response

DOI: 10.55415/deep-2022-0032.v1

element; TKIs, tyrosine kinase inhibitors; RhoA, ras homolog family member A; ROCK1, Rho-associated coiled-coil containing protein kinase 1; S6K, ribosomal protein S6 kinase; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; JNK, c-Jun N-terminal kinasea; UVA, ultraviolet A; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; ERK5, extracellular regulated protein kinases 5; IκB, inhibitor of NF-κB protein; GSH, glutathione; NSCLC, non-small cell lung cancer; NQO1, NAD(P)H quinone dehydrogenase 1; γ-GCS, γ-glutamylcysteine synthetase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; PPBMCs, Primary Peripheral Blood Mononuclear Cells; PFOS, perfluorooctane sulphonate; PD-1, programmed death-1; BTK, Bruton's tyrosine kinase; CDK4/6, cyclin dependent kinase 4/6; ALK, anaplastic lymphoma kinase; CSF3R, colony stimulating factor 3 receptors; BCSFB, blood–cerebrospinal fluid barrier; BBB, blood–brain barrier.

DOI: 10.55415/deep-2022-0032.v1

1. INTRODUCTION

Tumor is the consequence of uncontrolled proliferation related to cell cycle disorganization, where also involves inflammatory responses, apoptotic dysregulation, immune evasion and finally, metastasis [1, 2]. The battle with this malignance has been waged for centuries whereas this dreadful disease is still an important cause of death worldwide. The World Health Organization (WHO) announces that there are millions of people being confirmed the diagnosis of cancer across the globe every year [3-6]. Traditionally, four methods are used to combat cancer, including surgery, radiotherapy, chemotherapy, and immunotherapy alone or in combination [7]. Currently, for patients with advanced cancer, chemotherapy and radiotherapy are the leading therapy option whereas are limited by their serious side effects and unexpected resistance to chemotherapeutic drugs [8, 9]. Therefore, it's of great importance for the better management of this mortal disorder to unearth a novel treatment with minimal side effects, easy accessibility and low cost.

Nowadays, traditional medicinal herbs, plants, or their fruits containing beneficial bioactive constituents are still used by lots of the world population to maintain health and to prevent or treat diseases [10]. These bioactive constituents provide considerable strategies for discovering potential anti-tumor drugs [2,11]. Currently, most of anti-tumor agents generate from microorganisms, organisms and floras, such as Camptothecin (CPT) and Paclitaxel (Taxol®), tow well-known anti-cancer drugs derived from plant [10, 12].

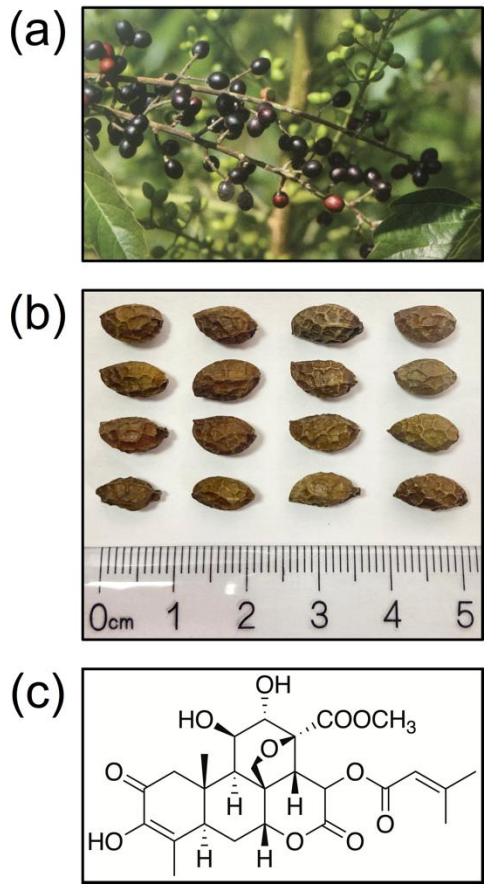
Brucea javanica (L.) Merr, growing extensively all over the southern China and southeast Asia, is a sort of indeciduous plant shrub from the Simaroubaceae family

DOI: 10.55415/deep-2022-0032.v1

[13]. The fruit of Brucea javanica (Figure 1a, b) has an ordinary breadth and length of about 5 mm and 8 mm respectively, with an oval-shaped and solid property [14]. As shown in the official Chinese Pharmacopoeia describing traditional Chinese medicine (TCM) components, the fruit of Brucea javanica is generally used to combat against lots of diseases, including diarrhea, malaria, intestinal inflammation and different kinds of cancer [15, 16]. In China, the Bruceae Fructus Oil is now used for the mangement of esophageal carcinoma, lung cancer and hepatocellular carcinoma clinically without toxic effects as well as with improved the function of immune regulation and the living quality of patients [17–20].

Brusatol (Figure 1c), a primary natural component of Bruceae Fructus, is regarded as one of the bioactive bases requisite for the anti-neoplasm property of Bruceae Fructus [21]. This product displays various biological effects, such as insecticidal activities [22], anti-malarial [23], anti-inflammatory [24], and anti-colitis [25, 26]. In addition, numerous efforts have been made for anti-tumor properties of this component, focusing on its immense possibility that it’s a promising anti-tumor drug.

DOI: 10.55415/deep-2022-0032.v1



In current work, we summarise the anti-tumor characters of Brusatol and its potential signaling pathways of effects in view of in vivo and in vitro experiments. Additionally, we summarise the toxicological studies, pharmacokinetics and drug delivery tool of Brusatol. What's more, based on the previous researches regarding Brusatol, we come up with some problems and suggestions, hoping to promote the study of Brusatol and provide people who interested in it a convenience.

2. RETRIEVAL AND ANALYSIS METHODS

A comprehensive retrieval was carried out to find all related articles published with the use of Brusatol using Web of Sciences (1994–present) and PubMed (1994–present). The retrieve strategy was that we tracked down all relevant research articles by using a keyword "Brusatol", and after reading all articles regarding

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Brusatol carefully, we concluded its details from the aspect of in vitro experiments and in vivo experiments.

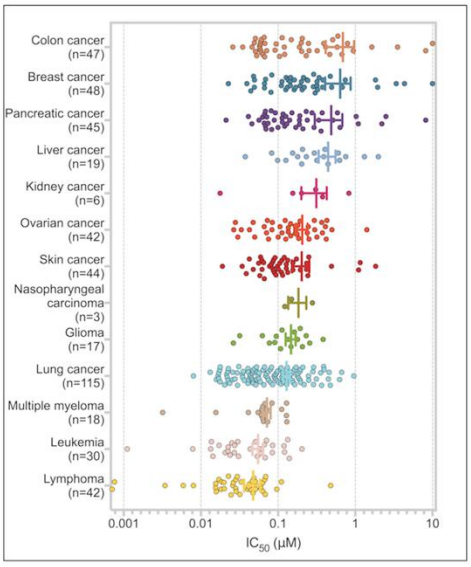
Among all the IC50 reported in the previous articles, we selected the data carried out with 72 hours Brusatol treatment, eliminated the data using problematic cell lines that had been demonstrated in the previous articles, grouped according to cancer types, eliminated the group with total samples less than three, and then performed all statistical analyses using Prism 7.0 software (GraphPad Software, USA). The results are shown as the mean ± SEM.

3. ANTICANCER PROPERTIES OF BRUSATOL

3.1. In vitro studies

In view of the in vitro experiments, Brusatol exhibited its potential to arrest cell cycle, promote apoptosis, induce autophagy, attenuate epithelial-mesenchymal transition (EMT), inhibit migration, invasion and angiogenesis as well as raise chemosensitivity and radiosensitivity using different cancer cell lines, as shown in Table 1. In addition, the half maximal inhibitory concentrations of Brusatol in cancer cell lines were shown in Figure 2, whose mean value in different kinds of cancer indicates the sensitivity of cancer to Brusatol to some extent. As shown in Figure 2, the sensitivity of cancer to Brusatol is in order of: Lymphoma > Leukemia > Multiple myeloma (MM) > Lung cancer > Glioma > Nasopharyngeal carcinoma (NPC) > Skin cancer > Ovarian cancer > Kidney cancer > Liver cancer > Pancreatic cancer > Breast cancer > Colon cancer.

DOI: 10.55415/deep-2022-0032.v1



3.1.1. Cell cycle arrest

The cell cycle, an essential process of cell division, mainly consists of four phases: pre-DNA synthesis (G1 phase), DNA synthesis phase (S phase), late DNA synthesis phase (G2 phase) and mitotic phase (S phase), among which Cyclin, Cyclin dependent kinases (CDKs) and Cyclin dependent kinase inhibitor (CKI) play an important role in regulation. CDKs, whose activity is dependent to its corresponding Cyclin, can promote cell cycle by phosphorylating its specific substrates. However, CKI can bind to CDKs thus inhibiting its kinase activity, and play a negative regulatory role in the cell cycle. [27-29] Cancer initiation or progression results from aberrant expression of various cell cycle proteins. Therefore, regulating cell cycle is becoming a promising target in cancer therapy [30, 31].

Brusatol induces cell cycle arrest at G0/G1, S or G2/M phase depending on the cell line character, not the cancer type. Among leukemia cell lines, Brusatol induced G0/G1 cycle arrest in Kasumi-1, K562, HL-60, Reh, SUPB13 and BV173, whereas caused metabolic arrest (S phase) in U937 and RS4;11. In addition, Brusatol can also

DOI: 10.55415/deep-2022-0032.v1

induce two phases (G0/G1 and S) arrest in leukemia cell lines BV173 at the same time. [32] Similar results were also found in NPC (CNE-1), with the decrease in Cyclin B1, Cyclin D1, Cdc2, and Cdc25c and the increment in p-Cdc2, the cell was also arrested not in just one phase. [33] In other cancer type, Brusatol also exerted anti-proliferation effect by interfering in cell cycle, including G0/G1 phase in PC9 and HCC827GRKU lung cancer cell lines [21, 34], Raji and SU-DHL-4 lymphoma cell lines [35] as well as G2/M phase in pancreatic cancer Capan-2 and PANC-1 cell lines [36]. In A375 melanoma cell, Wang et al. found that CyclinD1, CyclinE2, CDK4, and CDK6 were reduced following Brusatol treatment, thus causing G0/G1 cell cycle arrest [37]. These findings support that Brusatol could act as an inhibitor of the cell cycle in multiple tumor cells and then inhibit proliferation of tumor cells.

Based on the previous studies, the inhibitory effect of Brusatol on cell cycle should occur at most of stages, but the sensitivity of different stages of cell cycle to the inhibitory effect of Brusatol is different in various cancer cells. Therefore, the inhibitory effect of Brusatol is dominant at a certain stage in various cancer cells, whereas all the high expression proteins related to cell cycle may be inhibited.

3.1.2. Apoptosis

In multicellular organisms, apoptosis or programmed cell death, acts as a significant part in maintaining cellular homeostasis [38]. In brief, apoptotic pathways consist of two main pathways, including the mitochondrial-mediated pathway (intrinsic pathway) and the death receptor-mediated pathway (extrinsic pathway)[39]. The mitochondrial-mediated pathway is affiliated with alterations of mitochondrial

DOI: 10.55415/deep-2022-0032.v1

membrane permeability (MMP) which results in the unbalance of Bak and Bax thus releasing of mitochondrial Cytochrome C (Cyt C) together with other related proteins. The released Cyt C associates with apoptosis protease activating factor 1 (Apaf1) and then causes the formation of the apoptosome complex, which can activate Caspase-9 and later facilitate the downstream activation of Caspases-3 and -7 thus executing cell death. When it comes to the extrinsic pathway, the combination of ligands, including Fas ligand (Fas-L), tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand (TRAIL), to their corresponding receptors will activate Caspase-8 and induce apoptosis. [38–42] Notoriously, cancer cells avoid apoptosis and then make cancer initiation and development. Hence, it has become a major strategy in cancer therapy to promote cancer cells apoptosis [43].

Brusatol had shown up its capability of initiating Caspase-dependent apoptotic pathways in various cancer types. Zhang et al. found that MCF-7 human breast cancer cells lost their MMP when treated at 5μM of Brusatol, thus causing apoptosis, which was also confirmed in Bel-7402 human liver cancer cells [44]. The conclusion that Brusatol significantly caused the unbalance of Bcl-2 and Bax had also been demonstrated in other studies [21, 33, 37, 45-48]. The increasing Bax/Bcl-2 ratio resulted in the disengagement of Cyt C together with other related proteins, then causing the caspase cascade and apoptosis. Apart from this mitochondrial-mediated pathway (intrinsic pathway), Brusatol was capable of targeting tumor necrosis factor receptors (TNFR) and death receptors TNF-related apoptosis-inducing ligand (TRAIL)

DOI: 10.55415/deep-2022-0032.v1

receptors, thus causing caspase-8 and caspase -3 activated, then target proteins cleaved and finally apoptosis arising [21].

3.1.3. Autophagy

Physiologically, autophagy is a common procedure where cells that are under conditions of starvation or energy deficiency synthesize new ATP and macromolecules via a series of reactions [49]. Autophagy is regulated by mTORC complexes mTORC1 and mTORC2, especially mTORC1 whose activation can cause autophagy-related protein (ATG) phosphorylated and later autophagy inhibited [50, 51]. The curbs of mTORC1 will result in Unc-51-like autophagy-activating kinase (ULK) complex activated, causing the phagophore localized together with class III PI3K activated [52, 53]. Beclin-1 plays an important role in suppressing cancer during which it recruits some autophagosome elongation- and maturation-related proteins [54]. In addition, ATGs also act as a significant part in regulating autophagosome elongation. The microtubule associated protein 1 light chain 3 II (LC3 II) contributes to the combination of autophagosome with degraded substrates and then the autophagosome selectively removes damaged organelles using lysosomes. However, the disorder of ATG3 and ATG7 will interfere LC3 I to LC3 II and then the processes above. [55-57] Autophagy combats with various cancers by contributing to cancer cell death, which makes it a potent target for cancer treatment [58].

The potential of activating protective autophagy of Brusatol had been demonstrated in hepatocellular carcinoma (HCC) cell lines via the conversion of LC3 I to LC3 II, with autolysosomes forming and apoptosis arising. In Bel7404 cells, the

DOI: 10.55415/deep-2022-0032.v1

PI3K/AKT/mTOR signaling pathway was inactivated by Brusatol, thus causing cytoprotective autophagy. In addition, compared with the control group, this Brusatol-treated HCC cell line expressed less p62 but more Beclin-1. [45] These studies offer numerous evidences for Brusatol in the induction of cancer cells autophagy.

3.1.4. Epithelial-mesenchymal transition

EMT is a procedure during which the epithelial-like cells transform to migratory mesenchymal-like cells, involving in tissue fibrosis, tumor invasiveness, and metastasis. From the perspective of therapeutic depressions in cancer, the elasticity of this cellular characteristic offers a novel strategy. [59, 60] During the EMT, the mesenchymal proteins (N-cadherin and Vimentin) increase as well as the epithelial proteins (Cytokeratin and E-cadherin) lose [61]. In addition, some pivotal transcription factors (ZEB1/2, Snail1, Snail2 and Twist) also act as a significant role in EMT [59, 62].

Brusatol had been shown to suppress the EMT process in various kinds of tumors including gastric cancer, liver cancer, and lung cancer (Table 1). Brusatol suppressed the expression of mesenchymal proteins such as N-cadherin and Vimentin as well as matrix metalloproteinase (MMP)-9, inhibiting the invasion of gastric cancer cells [48]. In addition, the expression of epithelial marker E-cadherin and tight junction protein Occludin was also able to be induced by Brusatol and then inhibiting Fibronectin together with the inhibition of two key transcription factors Twist and Snail (Snail 1), thus suppressing the EMT procedure of HCCLM3 HCC cell line

DOI: 10.55415/deep-2022-0032.v1

[63]. Meanwhile, Brusatol could also decrease the expression levels of Slug and its target gene β-catenin in lung cancer. However, the expression of Slug and β-catenin is increased in A549 lung cancer when treated with Brusatol [64].

3.1.5. Migration, invasion and angiogenesis

As is known to us, involving the invasion and migration, metastasis is the leading death cause in patients with cancer. Nearby tissues are migrated and invaded by cancer cells, and by intruding to lymphatic or blood system, distant organs also. [65-67] Invadopodium of cancer cells, which comes into being via the stimulation of epidermal growth factor (EGF), is essential for remodeling membrane proteins and resolving extracellular matrix, thus causing metastasis promoted [65]. Hence, during this malignancy treatment, preventing the metastasis of cancer cells is one of the most significant businesses, for patients in early-stage in particular [67].

Numerous studies had shown that Brusatol had the capability to inhibit tumor metastasis in various kinds of cancer such as gastric cancer [48], hypopharyngeal carcinoma [68], liver cancer [45, 63], lung cancer [21,34, 64], oral cancer [68], and oropharyngeal carcinoma [68], which was often accompanied with the loss of epithelial markers and the increase of mesenchymal markers. Furthermore, Brusatol also showed its effect in inhibiting the expression of MMP-9, a kind of matrix metalloproteinases (MMPs) proteins, in gastric cancer cells, which played a core role in cancer metastatic process [48].

Angiogenesis, namely the formation of new blood vessels, can supply nutrients and oxygen for cells and tissues, which plays a significant part in the progression and

DOI: 10.55415/deep-2022-0032.v1

development of malignancies [69]. With the release of proteins promoting cellular growth and motility, the endothelial cells in developing cancer are roused and then conceive a net of blood vessels for the alleviation of hypoxia condition, among which vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF2) also play a core role [70, 71].

In oral cancer cell lines (JMAR and YD-10B), Brusatol down-regulated the expression of VEGF via the STAT3 pathway thus inhibited angiogenesis, which was also confirmed in hypopharyngeal carcinoma cells (UD-SCC-2 and FaDu) and oropharyngeal carcinoma cells (LN686) [68].

3.1.6. Chemosensitivity and Radiosensitivity

In the rapidly-dividing cells, radiation can cause the cleavage of water molecules and the generation of ROS, and then directly damage or injure the DNA, thus radiotherapy is used in cancer treatment generally [72]. Several factors impress the radiosensitivity of cancer cells, such as the therapy-altered pathways, the expression of proto-oncogenes or anti-oncogenes and the repair of DNA damage [73–75]. The drugs targeting these items are possible radio-sensitizers to therapy tumor, and numerous studies had shown that, during cancer radiotherapy process, Brusatol could markedly improve the effect.

Some researchers also observe the phenomenon where Brusatol could promote ROS production and enhance DNA damage through the inhibition of Nrf2 expression in A549 lung cancer cells in vitro when combined with radiotherapy [76]. In addition,

DOI: 10.55415/deep-2022-0032.v1

Brusatol had also been shown to inhibit the expression of Nrf2, p-ATR and p-CHK1, thus increasing radio-sensitivity [77].

With the unexpected adverse events and ultimate drug resistance, chemotherapy in cancer patients is limited currently. Thus, decreasing the side effects or increasing the sensitivity to chemotherapy is an important strategy for cancer therapy. In China, TCM herbal therapy has been regarded as a promising complementary therapy, for its doctrine of individualized therapy and conception of wholism to the different syndromes. In addition, it has numerous favorable effects for patients with cancer, such as prolonged overall survival, improved the living quality, and reduced adverse events. Chemotherapy combined with TCM therapy for cancer patients has gained more and more attention for the reason that TCM therapy attaches more importance to the overall functional adjustment and body recovery compared with chemotherapy. [78] Numerous researches have demonstrated that combination chemotherapy with TCM therapy can prolong overall survival of cancer patients by easing side effects of chemotherapy and enhancing personal immunity of patients, based on which, by using the bioactive components extracted from TCM, combination therapy for cancer patients is also attracting more and more attention to decrease toxicity and limitations of chemotherapy [79–82].

Several studies had demonstrated that, combined with Brusatol, chemotherapy had enhancive clinical effects with the reductive adverse events. Lots of studies had shown that Brusatol could increase the effect of many chemical drugs, including Trastuzumab, Taxol, Cisplatin (CDDP), Plumbagin, Thiabismocine, Irinotecan,

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Progestin, Cytarabine, Daunorubicin, Arsenic trioxide, 2,4-Dinitrochlorobenzene, Lodoacetamide, N-acetyl-p-benzoquinone imine, Paclitaxel, Erlotinib, Gefitinib, Afatinib, Osimertinib, Gemcitabine and 5-Fluorouracil [21, 34, 36, 83-91].

Moreover, in the human melanoma cells line A375, Brusatol significantly increased the effect of phototherapy by increasing ROS-induced cycle arrest and cellular apoptosis and inhibiting melanoma growth through the AKT-Nrf2 pathway [37].

3.2. In vivo studies

In view of the in vivo animal models, Brusatol exhibited its capability to inhibit tumor growth, invasion and metastasis, as well as promote apoptosis, radiosensitivity and chemosensitivity using various in vivo models, shown in Table 2.

The extent of suppressing tumor showed that Brusatol is markedly effective against different kinds of cancers in vivo, varying from 0–4 mg/kg through different injection means between injection into abdominal cavity or around tumors.

A lot of researches showed that Brusatol exhibited its effect of tumor growth inhibition by down-regulating the expression of Ki67 protein and up-regulating the number of TUNEL positive cells in colon cancer [93], glioma [94, 95], liver cancer [45], lung cancer [85], NPC [33], and pancreatic cancer [46]. More interestingly, pre-treatment with Brusatol contributed to cancer initiation whereas post-treatment with Brusatol prevented cancer progression in vinyl carbamate-induced carcinogenesis A/J mice.

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For years, the unexpected chemo- and radio-resistance have prevented the response of therapy to tumor. Brusatol enhanced the radiotherapy effect of ionizing radiation [77] and the chemotherapy effect of Trastuzumab [83], Irinotecan [89], CDDP [85, 96], Gemcitabine [36, 46], and 5-Fluorouracil [36].

Owing to the heavy brood, rapid maturation time and small size, Danio rerio (Zebrafish), as a complement against traditional cell models and mice tests, has recently become a significant cancer model. More importantly, its transparent body wall makes tumor progression visible and experiment labour alleviative. [97, 98] As is known to us, zebrafish embryo or juvenile zebrafish is used widely as a powerful tool to study cancer invasion, metastasis, and tumor-induced angiogenesis. According to Park S. et al.'s study, Brusatol reduced the number of lung cancer cells in drug-treated embryos and decreased the migration capability of lung cancer into body. What's more, compared with the control group, Brusatol reduced 55% area of cancer cells penetration in the vessels of the zebrafish larvae, which indicated that Brusatol was able to inhibit the cancer cells migration, invasiveness, and metastasis. [34]

4. EFFECT OF BRUSATOL IN DIFFERENT KINDS OF CANCERS

Numerous preclinical studies had offered evidences for the therapeutic potential of Brusatol and had shown its role in regulating different cancer hallmarks, such as proliferation, apoptosis, survival, invasion, metastasis and angiogenesis (Table 1 and 2). The effect of Brusatol in different kinds of cancers and its potential regulatory mechanisms of various proteins related to different kinds of malignancies are summarized below briefly.

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4.1. Breast Cancer

Breast cancer in women, surpassing lung cancer now, has become the dominant cause of incidence related to cancer worldwide with an approximate 2,300,000 new cases in 2020, accounting for 11.7% of all cases related to cancer, which is the fifth dominant cause of global death related to cancer with 685,000 deaths [99]. Zhang J. et al. demonstrated that Brusatol possessed great potential to promote cell apoptosis and inhibited cell viability via an oxidation pathway, which was significantly enhanced by redox-sensitive micelles [44]. In vivo and in vitro studies of breast cancer indicated that Brusatol displayed its anti-tumor capability by Inhibiting HER2-AKT/ERK1/2 and Nrf2/HO-1 pathways. At the same time, Brusatol could markedly enhance the effect of Trastuzumab by raising ROS accumulation and apoptosis level. [83] Similar studies also showed that Brusatol enhanced the effect of CDDP through the inhibition of Nrf2-mediated defense mechanism, suggesting that Brusatol was able to be developed into an adjuvant chemotherapy drug [85]. In addition, in murine ovarian cancer cell line 4T1, the Nrf2 inhibitor, Brusatol, could enhance the cytotoxic activity of Plumbagin through inhibition of the Nrf-2-mediated anti-oxidative response and mitochondrial electron transport [86]. Furthermore, Wu T. et al. demonstrated that Brusatol had the possibility as a novel chemotherapy-adjuvant drug to overcome refractory tumor initiating cancer stem cells (CSCs) [84].

4.2. Cervical cancer

In women, cervical cancer, with an approximate 604,000 new cases and 342,000 deaths worldwide, ranks fourth in terms of incidence and fourth in terms of

DOI: 10.55415/deep-2022-0032.v1

cancer-related mortality in 2020 [99]. Ren D. et al. demonstrated that Brusatol could enhance the effect of CDDP through the inhibition of Nrf2 expression in cell lines Hela [85].

4.3. Colon cancer

Colorectal cancer, with more than 1,900,000 new cases (including anus) and 935,000 deaths, accounting for approximate 1/10 cancer-related cases and deaths, is the third most frequently diagnosed cancer but the second dominant cause of cancer-related death in 2020 [99]. Lu Y. et al. reported that Brusatol decreased glucose consumption and down-regulated the expression levels of HIF-1α under hypoxia or CoCl2-induced hypoxia concentration-dependently in colon cancer cell line HCT116 without significant cytotoxicity, showing the HIF-1α regulation ability of Brusatol and suggesting its therapeutic possibility in colon cancers [100]. In another study, the combination of Brusatol and CDDP showed the synergistical inhibitory effect on cell proliferation and the synergistical increasing effect on cell apoptosis in CT-26 colon cancer cells [88]. Based on both in vivo and in vitro experiments, numerous evidences indicated that Brusatol was able to inhibit c-Myc/ROS signaling pathway, then increase HIF-1α through promotion of prolyl hydroxylases (PHDs) activity, and finally induce cancer cell death in color cancer under hypoxia [93]. In addition, the anti-cancer effect of Brusatol was also demonstrated in a syngeneic orthotopic mouse model of colorectal cancer [89]. Based on both in vivo and in vitro experiments, numerous evidences indicated that Brusatol was able to inhibit c-Myc/ROS pathway, then increase HIF-1α through promotion of

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prolyl hydroxylases (PHDs) activity, and finally induce cancer cell death in color cancer under hypoxia [93].

4.4. Endometrial cancer

In women, uterine corpus cancer, with 417,000 new cases and 97,000 deaths worldwide, ranks sixth in terms of incidence in 2020, among which the vast majority are of adenocarcinomas arising from the endometrium [99]. In the endometrial cancer cell lines Ishikawa and Spec-2, Brusatol could concentration-dependently decreased Nrf2 protein levels [85]. Kapur A. Et al. reported that Brusatol enhanced the cytotoxic activity of Plumbagin by araising oxidative stress [86]. In addition, Brusatol also could decrease progestin resistance through suppression of the Nrf2-AKR1C1 pathway [90].

4.5. Gastric cancer

Gastric cancer, with more than 1,000,000 new cases and an approximate 769,000 deaths worldwide (equating to 1/13 deaths), ranks fifth in terms of incidence and fourth in terms of mortality as well as is still a significant cancer in 2020 [99]. Chen H. et al. reported that Brusatol reversed lipopolysaccharide-induced EMT and induced apoptosis through PI3K/Akt/NF-κB pathway in human SGC-7901 gastric cancer cells [48].

4.6. Glioma

Brain cancer accounts for estimated 3% of all cancer-related cases worldwide [101]. In IDH1-mutated glioma, Brusatol exhibited a potent tumor suppressive effect

DOI: 10.55415/deep-2022-0032.v1

both in vitro and in vivo [94]. In another similar in vivo study, Brusatol inhibited IDH1-mutated cancer progression selectively [95].

4.7. Head and neck squamous cell carcinoma

With very poor prognosis, head and neck squamous cell carcinoma (HNSCC) is raising with a very fast rate every year in terms of incidence [102-104]. Lee J. et al. demonstrated Brusatol was a potential blocker of STAT3 pathway in various HNSCC cells, which reduced the STAT3 activation through abatement of its upstream kinases including Src, JAK1 and JAK2, and reduced the nuclear STAT3 levels and its combination ability with DNA [68].

4.8. Leukemia

Pei Y. et al. reported that Brusatol arrested G0/G1 cell cycle in vitro and suppress cancer growth in vivo [35]. Mata-Greenwood E. et al. showed that Brusatol could induce leukemic cell differentiation and G1 cycle arrest, which was related to down-regulation of c-myc [32]. In another similar study, Cuendeta M. et al. demonstrated that Brusatol-induced leukemic cell differentiation involved NF-kB activation in cell lines HL-60 [105]. In addition, Karathedath S. et al. reported that Brusatol in combination with chemotherapeutic agents such as Cytarabine, Daunorubicin and Arsenic trioxide could modulate drug resistance in acute myeloid leukemia [91].

4.9. Liver cancer

Primary liver cancer, with estimated 906,000 new cases and 830,000 deaths worldwide, ranks sixth in terms of incidence and third in terms of cancer-related

DOI: 10.55415/deep-2022-0032.v1

mortality in 2020 [99]. Zhang J. et al. showed that Brusatol decreased cancer cell viability via Nrf2/ARE signaling pathway [106]. In another study, Zhang J. et al. showed that Brusatol exhibited its anti-tumor effect via an oxidation pathway [44]. Ye R. et al. reported that Brusatol exhibited its anti-cancer effect via the PI3K/Akt/mTOR pathway, and then inhibited cell viability and promoted autophagy-caused apoptosis [45]. In addition, Lee J. et al. reported that Brusatol suppressed STAT3-driven metastasis via downregulation of EMT both in vitro and in vivo [63]. Furthermore, Brusatol prolonged the survival of H22 ascites tumor-bearing mice by inducing H22 cell apoptosis [107]. In mouse liver cancer cells Hepa-1c1c7, Brusatol provoked a fast and transient depression of Nrf2 expression and sensitized cells to chemical toxicity [108].

4.10. Lung cancer

Lung cancer, with an approximate 2,200,000 new cases and 1,800,000 deaths, accounting for estimated 1/10 (11.4%) cancers diagnosed and 1/5 (18.0%) deaths, ranks second in terms of incidence and first in terms of cancer-related mortality in 2020 [99]. Xie J. et al. demonstrated that the process that Brusatol inhibited proliferation in PC9 cell line might be intimately related to the inhibition of Nrf2-mediated antioxidant response and modulation of ROS-mediated mitochondrial-dependent pathway [21]. Park S. et al. demonstrated that Brusatol inhibited cancer cell proliferation as well as reversed Gefitinib resistance and EGFR-TKIs cross-resistance through inhibition of Nrf2 activity [34]. In another study, Brusatol enhanced the effect of chemotherapy via the inhibition of Nrf2-mediated

DOI: 10.55415/deep-2022-0032.v1

defense mechanism both in vitro and in vivo [85]. In addition, Sun X. et al. reported that Brusatol enhanced the radiosensitivity of A549 cell line through the promotion of ROS production and DNA damage [76]. In addition, Ko E. et al. reported that Brusatol decreased cell motility through RhoA–ROCK1 signaling [64].

4.11. Lymphoma and multiple myeloma

Brusatol arrested G0/G1 cell cycle by specifically targeting the PI3K/AKT pathway in Lymphoma [35]. In addition, Brusatol could suppress cell viability and growth by increasing oxidative stress in MM cells [109].

4.12. Nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC), an endemic malignancy in southeast Asia and southern China, is still a significant cause of death related to cancer globally, with an incidence of about 50,000 deaths every year [110, 111]. Based on in vivo and in vitro experiments, Brusatol induced apoptosis and inhibited growth in NPC cells, which involved the Akt/mTOR/S6K/4EBP1 pathway [33].

4.13. Ovarian cancer

Brusatol enhanced the cytotoxic activity of Plumbagin in ovarian cancer cell lines OVCAR3 and SKOV3 [86]. In another similar study, Brusatol enhanced anti-cancer ability of Trastuzumab both in vitro and in vivo [83]. In addition, Brusatol decreased CDDP resistance via the suppression of iron export related gene SLC40A1 in CoC1/DDP and A2780CP ovarian cancer cell lines [92].

4.14. Pancreatic cancer

DOI: 10.55415/deep-2022-0032.v1

Pancreatic cancer, with approximate 496,000 cases and 466,000 deaths worldwide, ranks seventh in terms of cancer-related mortality because of its poor prognosis in both sexes in 2020 [99]. Brusatol inhibited growth and induced apoptosis in pancreatic cancer via the suppression of JNK/p38 MAPK/NF-κb/Stat3/Bcl-2 pathway [47]. Brusatol enhanced the chemotherapeutic effect of Gemcitabine in pancreatic cancer by inhibiting the Nrf2 signaling pathway and increasing ROS accumulation in vitro and in vivo [46]. In addition, Lu Z. et al. demonstrated that Brusatol possessed potential cytotoxic effect in various kinds of pancreatic cancer cell with a favorable safety profile and enhanced anti-cancer effects of Gemcitabine and 5-Fluorouracil in in vivo and in vitro models, which were related to the suppression of EMT process [36].

4.15. Pheochromocytoma

In pheochromocytoma cell lines hpheo1, Brusatol decreased the expression of Nrf2 proteins when treated with a concentration of 40 nM. In addition, in murine pheochromocytoma cell lines MPC, Brusatol induced cell apoptosis and decreased colony formation by elevating level of reactive oxygen species (ROS) and accumulation of DNA oxidative damage. Furthermore, Brusatol suppressed liver metastasis of pheochromocytoma and prolonged overall survival of tumor-bearing animal in vivo. [112]

4.16. Skin cancer

In A375 human malignant melanoma cell line, Brusatol inhibited cell proliferation caused by G1 cycle arrest and triggered cell apoptosis through

DOI: 10.55415/deep-2022-0032.v1

downregulation of the Nrf2-mediated antioxidant response. In addition, Brusatol enhanced the phototherapy effect of UVA irradiation. [37]

5. EFFECT OF BRUSATOL IN DIFFERENT KINDS OF SIGNALING PATHWAYS

5.1. MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) pathway has a three-stage signaling process made up of MAPK, MAPK kinase (MAPKK), MAPKK kinase (MAPKKK), which are activated in turn and then regulate cell growth, inflammation, stress, differentiation or other important physiological or pathological effects. There are four main branch routes in MAPK signaling pathway, including ERK1/2, ERK5, p38 MAPK and JNK, among which p38 MAPK and JNK are related to growth, apoptosis and inflammation as well as ERK1/2 and ERK5 involve growth and differentiation. [113]

Brusatol suppressed HER2/ERK1/2 pathway (Figure 3) both in vitro and in vivo in human BT-474 breast cancer and SK-OV-3 ovarian cancer, thus enhancing the antitumor activity of Trastuzumab [83]. In Xiang Y. et al.' study, Brusatol inhibited growth and induced apoptosis via JNK and p38 MAPK pathway in both PANC-1 and PATU-8988 cells, which could be reversed by MAPK inhibitors SP600125 and SB203580 [47].

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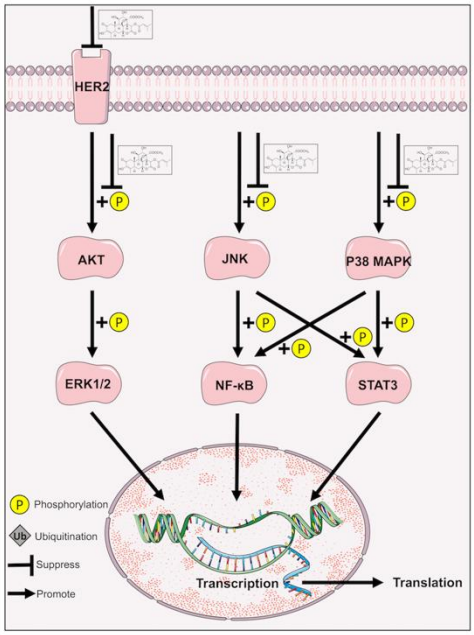


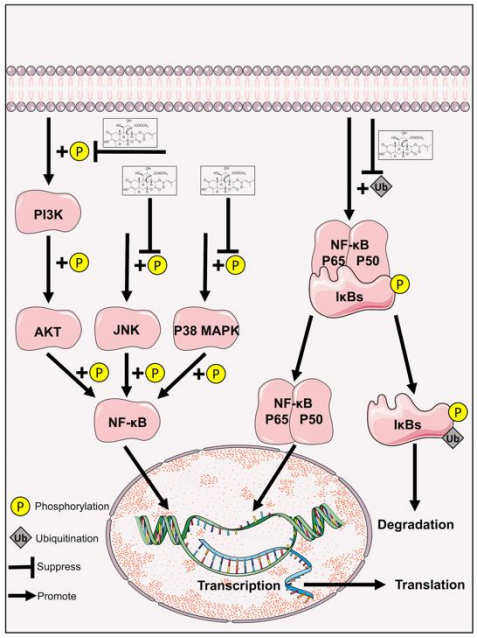
5.2. NF-κB signaling pathway

Nuclear factor kappa-B (NF-κB) protein is inactivated by binding to the NF-κB (IκB) inhibitor in the cytoplasm. Once the upstream signal factors bind to the membrane surface receptors, these receptors change their conformation and then transmit the signal to the IkB kinase, which increases the phosphorylation level of IkB and then dissociates it from the complex. Afterwards, the NF-κB quickly enters the nucleus from the cytoplasm and binds to the specific sequence on the nuclear DNA to promote the transcription of the related genes, so the continuous activation of this pathway will lead to the uncontrolled growth of the cells. Nowadays, targeting NF-κB is one of the hot topics in cancer therapy, whose main ideas are to inhibit the phosphorylation of IκB protein, the activity of NF-κB or the DNA binding activity of NF-κB. [114, 115]

Brusatol reversed lipopolysaccharide-induced EMT and induced apoptosis through NF-κB signaling pathway (Figure 4) in gastric cancer cell line SGC-7901

DOI: 10.55415/deep-2022-0032.v1

[48]. Brusatol deactivated NF-κB and arrested pancreatic cancer cell growth by inhibiting the Twist expression and stimulating the E-cadherin expression. In addition, Brusatol could also deactivate chemotherapeutic agents-induced NF-κB activation. [36] Cuendeta M. et al. demonstrated that Brusatol can induce differentiation in HL-60 cell line via NF- κB activation, involving p50 and p65, which could be inhibited by SN50, a NF-kB translocation inhibitor [105]. In addition, Xiang Y. et al. demonstrated that Brusatol induced apoptosis and inhibited growth by inhibiting NF-κB signaling pathway in cell lines both PANC-1 and PATU-8988, which could be attenuated by MAPK inhibitors SP600125 and SB203580 [47].



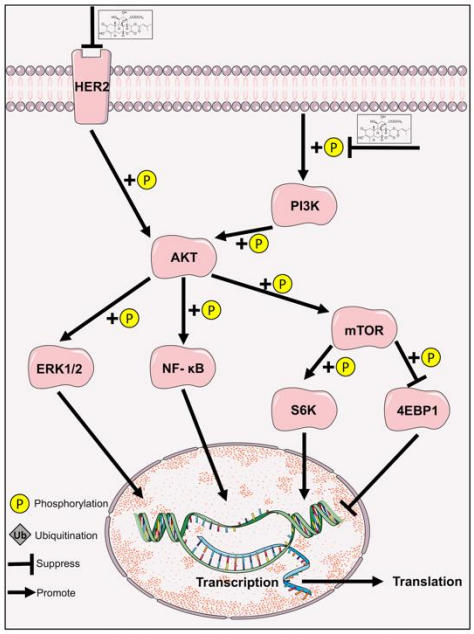
5.3. PI3K/AKT/mTOR signaling pathway

Mammalian target of rapamycin (mTOR), a threonine or serine protein kinase belonging to the phosphatidylin-ositol 3-kinase (PI3K)-related kinase family, plays a core role in pathways involved in cell growth and proliferation. [116] mTOR is regulated by a variety of cell signaling pathways, among which PI3K/Akt pathway is

DOI: 10.55415/deep-2022-0032.v1

the main pathway that transmits signals through mTOR and also acts as a significant role in mediating cell survival and proliferation [117-119].

Brusatol suppressed HER2/AKT signaling pathway (Figure 5) both in vitro and in vivo in human breast cancer BT-474 cell line and ovarian cancer SK-OV-3 cell line, thus enhancing the anti-tumor activity of Trastuzumab [83]. Brusatol reversed lipopolysaccharide-induced EMT and induced apoptosis through PI3K/Akt signaling pathway in SGC-7901 gastric cancer cell line [48]. Pei Y. et al. demonstrated that Brusatol specifically targeted the PI3K/AKT pathway in hematologic malignancy derived cells [35]. Ye R. et al. demonstrated that Brusatol exhibited anti-cancer effect by inhibiting cell viability and promoting of autophagy-caused apoptosis via the PI3K/Akt/mTOR signaling pathway in HCC [45]. In addition, Guo S. et al. demonstrated that Brusatol suppressed Akt/mTOR/S6K/4EBP1 protein synthesis signaling pathway and then contributed to anti-proliferation and pro-apoptosis effect in NPC [33].



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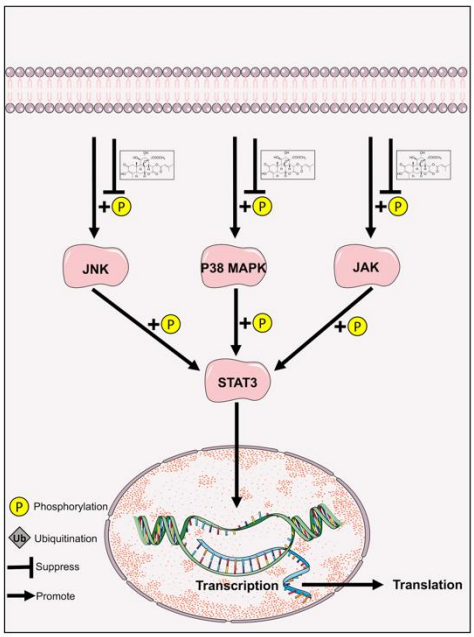
5.4. JAK/STAT signaling pathway

The well-known oncogene, signal transducers and activators of transcription (STATs), is regulated by G protein coupled receptors, receptor tyrosine kinases and interleukin families. The transcription factors STAT3 dimerize and translocate to mitochondria or nucleus after phosphorylation and then control cell cycle, cell growth, angiogenesis and cell survival, among which Janus tyrosine kinase (JAK), the upstream tyrosine kinase of STAT3, plays a significant role. [120, 121]

Brusatol inhibited growth and induced apoptosis by inhibiting Stat3 signaling pathway (Figure 6) in both PANC-1 and PATU-8988 cells, which could be attenuated by MAPK inhibitors SP600125 and SB203580 [47]. In addition, Brusatol suppressed STAT3-driven metastasis via the downregulation of EMT in HCC [63]. Furthermore, Brusatol inhibited JAK/STAT3 pathway in several HNSCC cell lines, thus intervening metastasis, angiogenesis, proliferation and apoptosis [68].



5.5. Keap1/Nrf2/ARE signaling pathway

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Under normal physiological conditions, Nrf2 are anchored by Keap1 in the cytoplasm. Once electrophiles or ROS attack the cells, Nrf2 protein dissociates from the Keap1, rapidly translocates into the nucleus, forms heterodimer with Maf protein, combines with antioxidant response element (ARE), and then activates Nrf2-mediated protein expression, including antioxidant enzymes, proteins related to GSH redox system, and proteins against drug resistance. [122, 123]

Brusatol induced apoptosis in non-small cell lung cancer (NSCLC) cell line via ROS-mediated mitochondrial-dependent signaling pathway and Nrf2/HO-1 signaling pathway (Figure 7) [21]. Brusatol suppressed Nrf2/HO-1 pathway both in vitro and in vivo in breast cancer BT-474 cell line and ovarian cancer SK-OV-3 cell line, thus enhancing the antitumor activity of Trastuzumab [83]. Brusatol enhanced the chemotherapeutic effect of Gemcitabine in pancreatic cancer via the suppression of Nrf2 pathway, which was associated with the down-regulation of antioxidant enzymes NQO1 and HO-1 [46]. The inhibition effect of Brusatol in antioxidant enzymes HO-1 and NQO1 was also showed in other Brusatol-related researches [84, 91, 94, 95, 124]. Brusatol could also interfere GSH redox system via Nrf2 Pathway, including reducing GSH, γ-GCS (rate-limiting enzymes for GSH biosynthesis), GCLC (catalytic subunits of γ-GCS) and GCLM (regulatory subunits of γ-GCS) [188], which was also showed in other Brusatol-related researches [84, 91, 94, 95, 106, 112].

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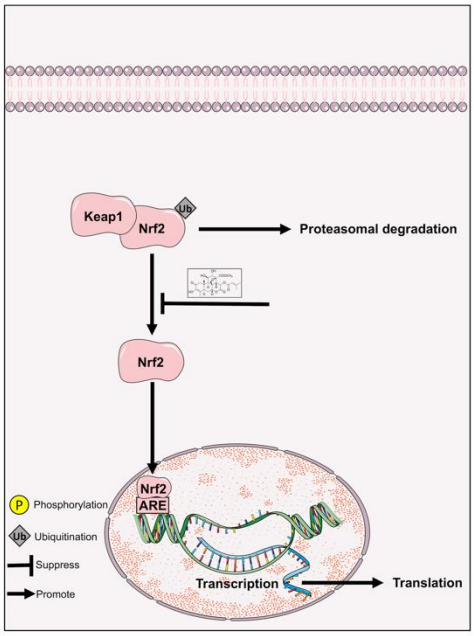

Aldoketo reductase family 1 member C1 (AKR1C1) is one of the Nrf2 target genes, whose leading function is to convert Progesterone to its inactive form [125, 126]. Brusatol reversed Progestin resistance by down-regulating the expression of AKR1C1 and Nrf2 in endometrial cancer [90]. The similar effect of Brusatol in AKR1C1 was showed in Park S.' research, which was associated with Gefitinib resistance and EGFR-TKIs cross-resistance [34].

6. TOXICOLOGICAL STUDIES

In cancer cells, Brusatol exerted more powerful cytotoxicity than many current chemotherapy drugs, such 5-Fluorouracil and Gemcitabine. But in human normal gastric epithelial GES-1 cells, Brusatol exerted more mild cytotoxicity than Gemcitabine and 5-Fluorouracil. The IC50 values of Brusatol/Gemcitabine were 11.6 (48 h) and 9.26 (72 h) as well as the IC50 values of Brusatol/5-Fluorouracil were 5.58 (48 h) and 4.39 (72 h) [36].

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In human normal breast HBL-100 cells, the IC50 value of Brusatol was 125.5 nM (72 h) [131]. Pei Y. et al. demonstrated that Brusatol inhibited protein synthesis with very small hematologic toxicity as seen in human normal Primary Peripheral Blood Mononuclear Cells (PPBMCs) [35]. In addition, a lot of researches had demonstrated that Brusatol can suppress brain cancer cells and reduce amyloid-β-, PFOS- and T-2 toxin-induced neurotoxicity [132-134]. But in human normal lymphoblastoid LCL1 cells, the IC50 value of Brusatol was 2.67nM (72 h) [35].

Numerous studies had declared that Brusatol ranging from 0–2 mg/kg exhibited anti-tumor effect with no significant toxicity in the tumor-bearing mice [33, 36, 45]. In addition, Lee J. et al. demonstrated that Brusatol treatment with high concentration of 5 or 10 mg/kg did not impart significant toxicity in experiment mice [63].

7. PHARMACOKINETICS OF BRUSATOL

Pharmacokinetics, including the time course of absorption, distribution, metabolism, and excretion (ADME), is a kind of drug movement studies in the body. In order to investigate Brusatol and furtherly extend its applications, it was essential to explained the in vivo process of Brusatol comprehensively. In terms of a new drug development, the research of in vivo process of Brusatol and detection of its metabolites will offer more valuable information for its anticancer mechanism and safety estimation.

In the previous researches, pharmacokinetics of Brusatol focused on three articles [127-129]. The researches have demonstrated that Brusatol undergone a fast absorption and distribution, but slowed metabolization after intravenous

DOI: 10.55415/deep-2022-0032.v1

administration [127-129]. The majority of Brusatol were excreted as metabolites and the major metabolic pathways of Brusatol in vivo were suggested by hydroxylation, hydrolysis and glucuronidation [127]. Regarding the distribution of Brusatol in vivo, the Brusatol peak concentration in lung was 10 folds or higher than other tissues (liver, kidney and spleen), suggesting that Brusatol maybe target at lung actively [128].

8. DRUG DELIVERY TOOL OF BRUSATOL

Owing to the low water solubility and bioavailability of Brusatol, Chen X. et al. developed a proper Brusatol delivery system using glycosaminoglycan-placental chondroitin sulfate A-modified nanoparticles to improve its pharmacological effectiveness, offering an effective and safe strategy for the treatment of various tumors [130].

9. FUTURE PERSPECTIVE

The cell line misidentification problem has been reported for decades whereas numerous articles using the wrong cells are still publishing without any warnings. In the biomedical sciences, this stubborn problem results in the irreproducible experiments, false conclusions and growing concerns about errors. It’s still insufficient to stop this cell line misidentification problem with decades-old and continuous efforts. [135-138] In this article, we marked problematic cell line demonstrated in the previous researches with “#” in table 1 and 2, calling for users and readers to interpret related papers with appropriate care.

In the previous researches, the effect and molecular mechanism of Brusatol on many kinds of cancer had been reported both in vitro and in vivo, whereas not in

DOI: 10.55415/deep-2022-0032.v1

bladder cancer, carcinoma of penis, carcinoma of vulva, esophageal carcinoma, osteosarcoma, prostate cancer and thyroid cancer. More studies are essential to claim the possibility and mechanism of Brusatol on these kinds of cancer, contributing to a more unambiguous anti-cancer profile.

Numerous studies had demonstrated that Brusatol inhibited the Nrf2 signaling pathway selectively. Nowadays, Brusatol are widely used as a Nrf2 inhibitor in many studies. In addition, some studies had also demonstrated that Brusatol can specifically target PI3K and STAT3. Based on the previous studies, we believe that there may be more targets of Brusatol for its excellent anti-tumor character. It is essential to test the character of Brusatol in other targets, such as EGFR, BTK, CDK4/6, ALK, Bcr-Abl and so on.

In the previous researches, pharmacokinetics of Brusatol only focused on intravenous administration whereas its cases are still unclear via intraperitoneal or oral administration. A lot of researches had reported that Brusatol suppressed tumor growth in vivo via the intraperitoneal route of administration. Thus, it was of great importance to investigate the in vivo process of Brusatol via intraperitoneal administration comprehensively, which might improve the understanding of its anticancer mechanism. In addition, as is known to us, oral administration of drugs is the most convenient, the most economical and safest clinically. However, pharmacokinetics and anti-tumor effect of Brusatol in vivo are still unclear via the oral route of administration. It would be more encouraging if Brusatol could exert its anti-tumor effect in vivo via oral administration. Furthermore, a lot of researches had

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demonstrated that Brusatol can suppress brain cancer cells and reduce amyloid-β-, PFOS- and T-2 toxin-induced neurotoxicity. However, it is also unclear whether Brusatol could pass through the blood–cerebrospinal fluid barrier (BCSFB) and blood–brain barrier (BBB) after these routes of administration when performed in mice.

Moreover, toxicological studies of Brusatol are still poorly few. It's significant to carry out more cytotoxicity test in normal tissue cells as well as acute and chronic toxicity test in animals, and then reveal the underlying mechanism of toxicological properties of Brusatol. At the same time, some drug toxicity indicators, such as safe dose, minimum toxic dose, maximum tolerated dose, and half-lethal dose, can provide a reference for subsequent preclinical and clinical trials.

Nowadays, novel drug delivery systems are very promising with advantages, such as increasing drug solubility, releasing drug slowly and delivering drug accurately [148]. However, there are few researches regarding Brusatol from this perspective.

Recently, immunotherapy is one of major hot topics in oncotherapy. Although some articles have demonstrated that Fructus Bruceae Oil can improve the immunity of patients, the study of the effects of Brusatol on immune system has not yet been carried out and is worth a try. It has not been reported whether Brusatol can enhance immune function against different cancers and sensitize the effect of the current immunotherapy in vivo.

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Although Brusatol has shown the powerful anticancer ability in different cancers, no clinical study has been carried out so far since the preclinical study of Brusatol has not been fully carried out. For the welfare of mankind, the researchers are expected to conduct more preclinical and clinical studies on the pharmacological properties of Brusatol, providing more understanding of Brusatol and finally bringing this constituent into the clinic.

10. CONCLUSIONS

For centuries, aiming to combat with cancer, investigators expend enormous efforts for more treatment strategies through various approaches. In different kinds of cancer cell models, Brusatol, a primary natural constituent of Bruceae Fructus, exerts multiple effects such as cell cycle arrest regulation, autophagy induction, apoptosis induction, angiogenesis suppression and metastasis inhibition via different kinds of signaling pathways. What's more, in in vivo animal models, its anti-tumor effects are also confirmed with promising results of inhibiting tumor growth and prolonging animal survival. This current review describes in detail and discusses the various reports supporting the possibility of Brusatol to be a hopeful drug candidate for cancer therapeutics.

SUPPORTING INFORMATION

Table S1: The detailed information of "Problematic cell line" or "Caution" in in vitro experiments, Table S2: The detailed information of "Problematic cell line" or "Caution" in in vivo tumor bearing animal models, Table S3: More information (used

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concentration) in in vitro experiments, Table S4: More information (cell number, site of tumor xenograft, dose, frequency, duration, route of administration) in in vivo tumor bearing animal models.

ACKNOWLEDGMENTS

This research was supported by the National College Students' Innovation Entrepreneurship Training Plan Program of China (No. 201910570006), the Special Funds for the Cultivation of Guangdong College Students' Scientific and Technological Innovation ("Climbing Program" Special Funds) (No. pdjh2019b0403), the Guangzhou Medical University College Students Science Technology Innovation Project (No. 2018A019).

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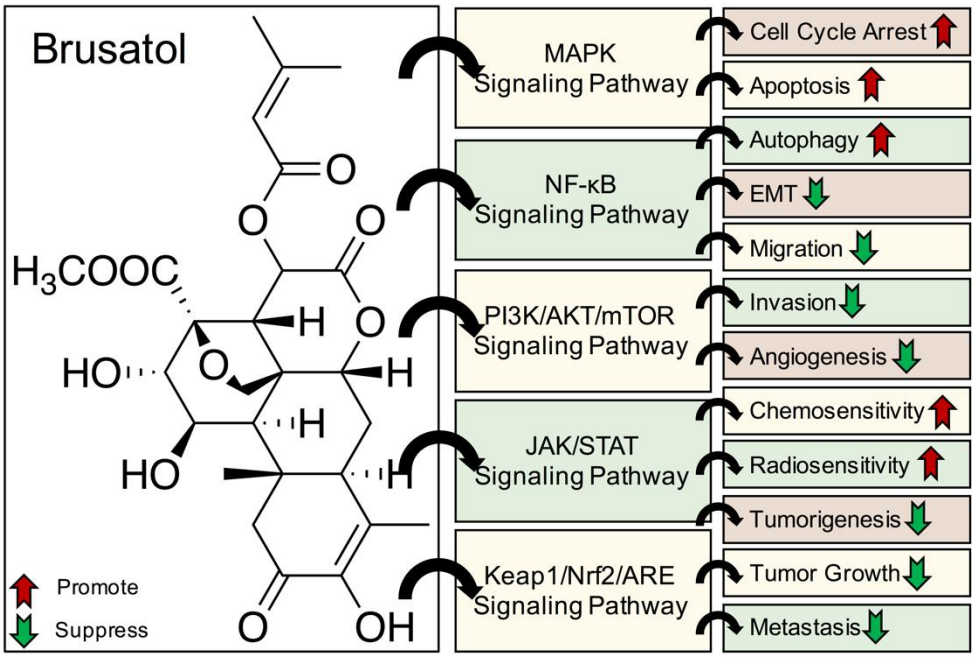
GRAPHICAL ABSTRACT



Highlinghts

1. Brusatol shows excellent efficacy against different kinds of cancers.

2. Brusatol exhibits its anti-cancer capability by numerous effects.

3. The anti-cancer effect of Brusatol involves various oncogenic signaling pathways.

4. Brusatol is a hopeful drug candidate for cancer therapeutics.

5. Based on the previous researches regarding Brusatol, we come up with some problems and suggestions in this article.

Table Legends

Table 1 | The antitumor effects of Bruastol against cancer cells in in vitro experiments

Table 2 | The anticancer effects of Brusatol in in vivo tumor bearing animal models

DOI: 10.55415/deep-2022-0032.v1

Figure Legends

Figure 1 | **a**) The fruits of Brucea javanica (from *The Plants in Shaoguan National Forest Park*, ISBN: 978-7-5219-0150-4). **b**) The sundried fruits of Brucea javanica. **c**) The structure of Brusatol.

Figure 2 | The effect of Brusatol in different kinds of cancers. The results are presented as the mean ± SEM.

Figure 3 | The effect of Brusatol in the MAPK signaling pathway.

Figure 4 | The effect of Brusatol in the NF-κB signaling pathway.

Figure 5 | The effect of Brusatol in the PI3K/AKT/mTOR signaling pathway.

Figure 6 | The effect of Brusatol in the JAK/STAT signaling pathway.

Figure 7 | The effect of Brusatol in the Keap1/Nrf2/ARE signaling pathway.