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Emerging Antitumor Activities of the Bitter Melon (*Momordica charantia*)

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Abstract

Bitter melon or bitter gourd (*Momordica charantia*) is a common vegetable in Asia and it is distinctive for its bitter taste. As an ingredient in folk medicine, research from different laboratories in recent years supports its potential medicinal applications with anti-tumor, anti-diabetic, anti-HIV activities in both *in vitro* and animal studies. In this short review, we summarize herein the recent progress in the antitumor aspect of bitter melon with a focus on the underlying molecular mechanisms. Further mechanistic studies as well as clinical trials are necessary to further verify its medicinal applications.

Keywords

Momordica charantia; bitter melon; anticancer; apoptosis; DNA damage; medicinal applications

1. INTRODUCTION

Natural products were the pre-eminent source of drugs or drug additives before the 20th century, and have continued to be an invaluable hub for new drug candidates [1, 2]. From 1981 to 2002, out of a total of 877 small-molecule new chemical entities, 49% were natural compounds or derivatives of natural compounds [3]. The advanced techniques of the modern drug discovery machine allow for accelerated drug discovery in natural products [1, 4].

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

DISCLAIMER

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CONSENT FOR PUBLICATION

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We have been working for 30 years on bitter melon (*Momordica charantia*) as a drug candidate [5–12]. Belonging to the family of *Cucurbitaceae*, this herbaceous tropical and subtropical vine is extensively cultivated in numerous Asian countries (especially China, India, Japan, and Malaysia), East Africa, the Caribbean, the Amazon, and also some parts of South America. It is also vernacularly named as bitter melon, bitter squash, balsam pear, karela, bitter apple, or wild cucumber in different countries [5]. Research from our laboratory and others has shown that bitter melon contains over 30 medicinal natural products, including proteins, peptides, and small compounds, and some of them exhibit antitumor, anti-diabetic, and anti-HIV potentials. Recently, we have published several bitter melon reviews, focusing on the activities and molecular mechanisms of its anti-tumor, antidiabetic and anti-HIV activities [5, 6, 11]. To avoid duplication and overlap, we herein focus on the latest outputs regarding the multiple antitumor molecular mechanisms of these products which were briefly touched upon in the above papers. We divide the review into results on antitumor activity obtained on different compounds originating from the bitter melon fruit. We start with crude extracts and then proceed to the purified compounds.

2. CRUDE EXTRACTS

In 1983 it was reported that bitter melon extract had antitumor activity in mice [13]. A number of more recent studies have looked at the anti-tumor potential of the crude extract of bitter melon. Cell proliferation was inhibited by bitter melon extract in breast cancer cell lines (MDA-MB-231, MCF-7), colon cancer cell lines (HT-29, SW480), and pancreatic cancer cell lines (BxPC-3, MiaPaCa-2, AsPC-1) [14–16]. All cell lines were inhibited in a dose-dependent manner after incubation for 48 hours [14–16]. Ray *et al.* observed more than 80% cell death in breast cancer cells treated with 2% bitter melon extract (Chinese variety, extracted using household juicer) [14]. Very recently, bitter melon juice also showed antitumor activity against gemcitabine-resistant pancreatic cancer cells [17].

The mechanism of the anti-tumor activity of bitter melon crude extract varies depending on the type of crude extract, the cell type, and how the crude extract is prepared. Both apoptosis and autophagy have been shown to be pathways of bitter melon-mediated cell death [14–16]. Ray *et al.* observed breast cancer cell death by apoptosis, as shown by PARP cleavage and caspase activation of MCF-7 and MDA-MB-231 cells treated with varying concentrations of bitter melon extract [14]. In the same study, Ray *et al.* found that survivin, XIAP, and claspin, which all contributed to an increase in cell division, were inhibited in breast cancer cell lines treated with 2% bitter melon extract [14]. In addition, catalase was up-regulated, while Bcl-2 and cIAP-1 were inhibited, further suggesting cell death by apoptosis in breast cancer cell lines [14]. Similarly, in pancreatic carcinoma cell lines BxPC-3 and MiaPaCa-2 treated with bitter melon extract (Chinese variety deseeded and juiced with household juicer, centrifuged and pellet discarded), caspase-3 and caspase-9 were activated [16]. The bitter melon extract also down-regulated XIAP and survivin levels, similar to what was seen in the breast cancer cell lines [16]. Kaur *et al.* suggested an intrinsic apoptotic mechanism based on an enhancement of p21, CHOP, and phosphorylated p38, as well as the release of cytochrome c in pancreatic cancer cell lines treated with bitter melon extract [16].

In contrast, Kwatra *et al.* found that bitter melon extract (methanol extract of both skin and whole fruit) did not induce apoptosis in colon cancer cell lines [15]. Instead, they suggested autophagy as the mode of colon cancer cell death, as cells treated with bitter melon extract showed autophagic vacuoles, confirmed by immunofluorescent studies as well as monodansylcadaverine measurements and Beclin-1 and Bcl2 levels. In addition, an increase in Atg 7 and Atg 12 showed cell death was mediated by autophagy after treatment with bitter melon extract [15]. Autophagy was induced via an AMPK-mediated pathway, as shown by an ATP decline and in AICAR measurements. When ATP was added, suppression of proliferation ceased in a dose-dependent manner. A decrease in phosphorylation of mTOR and p70S6K also suggests an AMPK pathway facilitated by mTOR [15].

Interestingly, Kaur *et al.* also found activated AMPK played an important role in apoptotic cell death [16]. Bitter melon extract was found to activate AMPK in three pancreatic cancer cell lines [16]. In addition, Kaur *et al.* used Compound C to show the important role AMPK plays in apoptotic death of pancreatic cancer cells treated with bitter melon extract [16]. When Compound C was added, which inhibits AMPK, caspase-3 activation was attenuated [16]. Therefore, bitter melon extract activated the AMPK pathway in several cancer cell lines, progressing towards either autophagic or apoptotic cell death depending on cell origin. It should also be noted that the methods of extraction used in different studies were different, which may also contribute to differences in the mechanisms of cell death.

Bitter melon crude extract has also been shown to arrest the cell cycle [14–16]. Breast cancer cell line MCF-7 treated with bitter melon extract showed cell cycle arrest at the G2-M phase, as suggested by FACS analysis [14]. In one type of colon cancer cell line, HT-29, bitter melon extract also arrested the cell cycle in the G2-M phase [15]. Interestingly, bitter melon extract did not affect the G2-M phase of the other colon cancer cell line, SW480, but instead induced an S-phase arrest [15]. All colon cancer cell cycle arrests were seen in a dose-dependent manner and observed by flow cytometry after 24 hours of exposure to the extract [15].

Cancer stem cells have recently entered the spotlight as they are oftentimes responsible for patient relapses [15]. These cells display distinct markers including DCLK1 and Lgr5 expression. The prevalence of colon cancer stem cells, as detected by the presence of these markers through flow cytometry, was decreased by bitter melon extract [15]. The ability of the extract to inhibit ca [15].

In addition to *in vitro* studies, Kaur *et al.* examined the effects of bitter melon extract on pancreatic cancer xenograft volume in mice [16]. These *in vivo* studies showed a strong decrease in MiaPaCa-2 tumor weight in athymic nude mice, without any associated toxicity [16]. Bitter melon crude extract affects a variety of cancerous cells in various ways. A number of factors contribute to its anti-tumor effects, which vary depending on the cell type and how the extract itself is prepared.

3. MOMORDICA CHARANTIA LECTIN (MCL)

Momordica charantia lectin (MCL) is a type II Ribosome Inactivating Protein (RIP), known to be particularly toxic, yet useful as an anti-tumor agent [17, 18]. Type II RIPs generally have two chains, each consisting of 261 amino acids [18]. MCL has been found to inhibit cell viability and induce autophagy, apoptosis, and cell cycle arrest [7, 18].

MCL was shown to decrease cell viability in nasopharyngeal Carcinoma (NPC) cell lines CNE-1 and CNE-2 [18]. Likewise, MCL inhibited the growth of hepatocellular carcinoma (HCC) cell lines HepG2 and PLC/PRF/5 *in vitro* in a dose- and time-dependent manner as shown by MTT analysis and colony formation assay [7]. Mechanistically, HCC cells demonstrated both autophagy and apoptosis in response to MCL. NPC cells were not evaluated for autophagy, but apoptosis was apparent.

MCL induced apoptosis in both HCC cells and NPC cells [7, 18]. In HCC cells, an increase in apoptotic cells was detected as evidenced by Annexin V/PI staining, chromatin condensation, DNA fragmentation, Bid activation and PARP cleavage [7]. In CNE-1 and CNE-2 NPC cells, a dose-dependent increase in apoptotic cells was also observed upon exposure to MCL [18]. Exposure to MCL activated caspases 8 and 9 in HCC cells, suggesting participation of both intrinsic and extrinsic pathways of apoptosis [7]. Further experimentation with HCC cells showed that induction of apoptosis was also involved the MAPK pathway [7]. Both p38 and JNK, downstream kinases of MAPK, are involved in regulation and phosphorylation of Bim, and Bim itself is associated with the IC50 of MCL in HCC cells [7]. In addition, p38 cleaves Bid, leading to apoptosis [7]. The phosphorylation of both p38 and JNK increased upon exposure to HCC cells treated with MCL [7]. In NPC cells treated with MCL, an increase in phospho-p38 was also seen [18]. In addition, there appeared to be a significant increase in JNK phosphorylation levels after exposure to MCL for 24 hours [18]. However, there was no effect on Bid, Bak, or BCL-2 in NPC cells [18].

After exposure to MCL, the cell cycle was arrested in both HCC and NPC cells [7, 18]. Flow cytometry showed that MCL induced a G2/M phase arrest in HCC cells [7]. In addition, Western blot analysis showed an increase in p21 after exposure to MCL [7]. However, NPC cells were arrested in G1 phase rather than G2/M phase (also shown by flow cytometry) [18].

MCL also displayed anti-tumor effects *in vivo* in both HCC and NPC cells [7, 18]. HCC mice treated with MCL three times per week showed significantly less tumor growth [7]. On day 22, tumor growth was halted by 53.6% [7]. Similarly, in CNE-2 xenograft tumors, by day 16 of MCL treatment, a 45% reduction of tumor volume was observed [18]. Toxicity to the treated mice was not observed in either study [7, 18]. In addition, HCC cells treated with MCL in combination with sorafenib showed much stronger anti-tumor effects than either MCL or sorafenib alone [7]. When tested in NPC cells, the combined administration of MCL and sorafenib showed much promise than the individual treatments [7].

4. MOMORDICA ANTIVIRAL PROTEIN 30 (MAP30)

MAP30 is a single-chain RIP with strong anti-tumor potential similar to MCL [9, 19]. The National Cancer Institute tested MAP30 on 9 cancer types and found inhibition in brain, breast, melanoma, and renal tumor cell lines. The MAP30 protein consists of 286 amino acids and the mature protein contains one N-glycosylation site. Specifically, MAP30 acts as a type I RIP, a glycosylase that aids in the binding of elongation factors [20]. DNA topological inactivation and DNA glycosylase/apurinic lysase activity are believed to be of particular importance in its anti-tumor activity. Like MCL, MAP30's anti-tumor activity has been shown to inhibit cancer cell line proliferation and induce apoptosis and arrest of the cell cycle [9, 20].

Fang *et al.* performed studies examining the effects of MAP30 (purified in accordance with the procedure used by Lee Huang *et al.*) on liver cancer cell line, HepG2 [9]. They found that MAP30 inhibited HepG2 proliferation using an MTT assay [9]. Meanwhile, Fan *et al.* showed dose- and time-dependent inhibition of colorectal carcinoma LoVo cells, similar to what was seen in the HepG2 cells [19] using recombinant MAP30, generated via a cDNA fragment amplified by RT-PCR, which was then cloned and confirmed by sequencing [19]. Both purified and recombinant MAP30 induced cell cycle arrest of HepG2 and LoVo cell lines respectively [9, 19].

Similar to other bitter melon components, Annexin V/PI double staining showed purified MAP30 induced apoptosis in Hep-G2 cells [9]. Other apoptotic features were detected within the cells similar to what was observed with MCL [9]. Flow cytometry showed recombinant MAP30 induced apoptosis in LoVo cells as well [19]. The apoptotic cell population initially accounted for 4.2% of the total and increased to 71.5% upon treatment with 4 μ M MAP30 for 24 hours [19].

The apoptotic pathway was further examined in both cell lines. A decrease in p23 was observed via Western blotting, while an activation of caspase -8, -9, and -3 and cleavage of PARP were also observed in Hep-G2 cells treated with purified MAP30 [9]. Surprisingly, there did not appear to be any effect on Bak or Bid levels, which are both involved in promoting apoptosis [9]. JC-1 staining showed MAP30 increased mitochondrial membrane depolarization, which allows caspase-9 to enter the nucleus and activate [9]. While phosphorylation of JNK (a MAPK with proapoptotic potential) was unaffected by MAP30 treatments, there did appear to be an increase in phosphorylation of other MAPKs (p38 and ERK), which were then shown to be essential to MAP30-induced apoptosis [9]. Fan *et al.* noticed a decrease in the Bcl-2 expression in LoVo cells treated with MAP30, indicating a down-regulation of Bcl-2 may contribute to MAP30-induced apoptosis [19]. Meanwhile, Bax was down-regulated when LoVo cells were treated with MAP30 [19]. Both down-regulation of Bax and up-regulation of Bcl-2 may together be essential components of MAP30-induced apoptosis in LoVo cells [19].

In vivo trials of MAP30 were consistent with those *in vitro*. Using breast cancer xenografts, MAP30 treatment increased survival of mice from 35 days to 64 days, without any toxicity to the mice. Similarly, tumor weight and volume were significantly decreased in nude mice

with HepG2 xenograft tumors treated with 2 mg/kg injections every other day [9]. Thus far, MAP30 shows promising potential as a powerful anti-tumor agent.

5. ALPHA-MOMORCHARIN AND BETA-MOMORCHARIN

Like MAP30, both α -momorcharin (α -MMC) and β -momorcharin (β -MMC) are type I RIPs, containing only one enzymatic chain [5, 8, 21]. α -MMC is a 30-kDa glycoprotein, while β -MMC is slightly smaller at 29-kDa [5]. Both have anti-tumor activity individually, as well as when purified from bitter melon together as MCP30 [8]. Both α -MMC as well as MCP30 induced apoptosis and cell cycle arrest in NPC cell lines and prostate cancer cell lines, respectively [8]. While α -MMC displayed cytotoxic effect on NPC cell lines CNE-1 and HONE1, it showed very little cytotoxic effects to non-cancerous transformed human nasopharyngeal epithelial NP69 cells [8].

Hypoxia is particularly dangerous because it can often lead to metastasis and resistance to chemotherapy [8]. Unfortunately, hypoxic regions are very common in NPC cells [8]. α -MMC is of particular interest because it has powerful potential in its ability to target hypoxic factors in tumors [8]. In NPC cell lines, it is common to find an overexpression of HIF1 α and VEGF, making them therapeutic targets [8]. Both HIF1 α and VEGF are associated with tumor hypoxia and promote angiogenesis and metastasis [8].

NPC cells CNE-2 and HONE1 were pre-treated with cobalt chloride to induce hypoxia (as occurs naturally *in vivo*), causing an increase in both HIF1 α and VEGF levels [8]. Upon α -MMC treatment, both HIF1 α and VEGF levels were decreased [8]. Human umbilical vein endothelial cells (HU-VEC), which are a key component to angiogenesis, were also down-regulated [8]. In addition, immunoblotting analysis revealed that NPC cells treated with α -MMC down-regulated unfolded protein response (UPR), which tumor cells in hypoxia rely on for survival [8]. IRE-1, a contributing pathway to the overarching UPR, was also down-regulated upon α -MMC treatment [8]. It is important to note that α -MMC's anti-tumor potential is potent in hypoxic regions, as they are particularly dangerous and lead to metastasis and resistance to chemotherapy [8].

Like other bitter melon components, Annexin V/PI staining showed that α -MMC induced apoptosis in both CNE-2 and HONE1 cells [8]. In CNE-2 cells, Western blot analysis showed activation of caspase -8, -9, and -3 in a time- and dose-dependent manner, characteristic of apoptosis [8]. However, caspase activation was very slight in HONE1 cells [8]. CNE-2 cells showed cell cycle arrest in G0/G1 phase, while HONE1 cells were arrested in S-phase upon α -MMC treatment [8]. Pan *et al.* thus stated that α -MMC anti-tumor potential works by inducing cell cycle arrest and apoptosis [8].

The anti-tumor potential of α -MMC in conjunction with β -MMC as MCP30 was also investigated by Xiong and colleagues [21]. MCP30 (purified from bitter melon according to methods described by Barbieri *et al.*, Lee-Huang *et al.*, and Ye *et al.*) induced apoptosis in prostate cancer cell lines PC-3, LNCaP, and PIN, but had no cytotoxic effect on noncancerous prostate cell line RWPE-1 [21]. FACS and Western Blot analysis confirmed tumor growth inhibition was due to induction of apoptosis and cell cycle arrest [21]. *In vivo*,

MCP30 treatment twice a week significantly inhibited tumor growth with significant differences in TUNEL staining as well, indicating apoptotic induction of PCa tumor cells [21].

Interestingly, Xiong *et al.* found that MCP30 decreased histone deacetylase (HDAC) activity [21]. HDACs are known to interact with oncogenic fusion proteins and are overexpressed in several tumor types [21]. MCP30 selectively increased histone acetylation (overall H3 and H4 level remained unchanged, while histone 3 and 4 acetylation increased) in PC-3, LNCaP and PIN cell lines [21]. Xiong *et al.* mentioned that hypo-acetylation of Histone-H4 is common in tumors [21].

Xiong *et al.* noted PTEN (a tumor suppressor gene) was expressed at high levels in normal prostate cell lines, while PIN cell lines contained very low amounts, and PCA cell lines expressed no detectable PTEN [21]. After treatment with MCP30, PTEN expression significantly increased in both prostate cancer cell lines, with no long-term effect on normal prostate cell lines [21]. In addition, PIN and PCa cell lines showed high β -catenin levels, a protein responsible for regulating the Wnt pathway, which indirectly stimulates cellular proliferation [21]. MCP30 treatment promoted β - catenin degradation, in turn suppressing Wnt signaling [21]. A decrease in cyclinD1 and c-MYC (which are induced due to Wnt activation) was also measured after MCP30 treatment [21].

6. RIBONUCLEASE MC2 (RNASE MC2)

Ribonucleases (RNases) degrade RNA [22]. Until recently, the only RNase purified from bitter melon was RNase MC1, with a molecular weight of 21 kDa [22]. In 2011, Fang *et al.* purified and characterized RNase MC2, a second ribonuclease from bitter melon with a molecular weight of 14 kDa [22]. Its cleavage activity was shown on tRNA in baker's yeast, calf liver, and rRNAs from MCF-7 breast cancer cells [22]. Bioinformatics showed no sequence similarity with other known RNases [22]. RNase MC2 was shown to be cytostatic and induce cell death in MCF-7 cells, suggesting its potential as yet another anti-tumor agent from bitter melon [22].

Flow cytometry showed RNase MC2 induced apoptosis in a dose-dependent manner in MCF-7 cells [22]. Other characteristic features of apoptosis were also observed [22]. Monitoring phosphorylation levels showed that RNase MC2 treatment activated Akt, p38, JNK1, and ERK [22]. Phosphorylation of Akt, p38, and JNK1 is known for proapoptotic activity, while ERK phosphorylation can be either pro-apoptotic or involved in tumor progression [22]. In addition, both caspase 8 (extrinsic pathway) and caspase 9 (intrinsic pathway) cascades were activated upon treatment [22]. Both pathways activate caspase 3 and induce apoptosis [22]. Finally, PARP was cleaved and Bak levels were increased, which are pro-apoptotic activities [22]. However, no changes were observed in the levels of pro-apoptotic Bid [22].

The anti-tumor potential of RNase MC2 was also tested in liver cancer cell line HepG2 [10]. MTT assay showed RNase MC2 decreased viability of HepG2 cells with cell cycle arrest in S-phase [10]. Annexin V/PI staining and TUNEL assay suggested apoptosis was induced in

HepG2 cells treated with RNase MC2 [10]. Similar to what was seen in MCF-7 cells, RNase MC2 treatment increased levels of Bak, activation of caspases 8 and 9, and induced PARP cleavage, triggering apoptosis [10, 22]. Again, RNase MC2 treatment activated phosphorylation of Akt, p38, JNK, and ERK in HepG2 cells, just as was seen in MCF-7 cells [10, 22].

The effects of RNase MC2 on HepG2 cells were also tested *in vivo* [10]. After 2.0 mg/kg RNase MC2 injections every other day, treated mice showed smaller hepatocellular carcinoma xenograft tumor weight than control mice [10]. H&E staining showed apoptosis was induced in the treated mice [10]. RNase MC2 is a promising anti-tumor agent, especially given its continued potency *in vivo* [10].

CONCLUSION AND FUTURE PERSPECTIVES

The type 1 RIPs, type 2 RIP, lectin and RNase in bitter melon are potential therapeutic agents for the treatment of cancer. They induce apoptosis in various types of cancer cells and the apoptotic pathways have been elucidated in many cases. More cancers may be revealed to be responsive to these bitter melon proteins by further research. Immunotoxins based on RIPs have been employed to target against hematological malignancies. Mistletoe lectin, a type 2 RIP, has been used clinically to improve the quality of life in cancer patients. Onconase, an amphibian lectin and RNase, has anticancer activity in patients. The same may be true of the bitter melon proteins. Besides proteins, there is a variety of non-proteinaceous small molecules with important biological activities in the bitter melon. It is of little surprise that the bitter melon is widely used in traditional medicine. Since most of the data on antitumor activity summarized here were limited to those derived from studies employing patient cells and laboratory nude mouse models and the actual potential toxicity of them are not available, more basic laboratory studies of promising components should be performed before moving on to clinical trials.

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Fig. (1). Possible mechanisms of the anti-tumor effects of bitter melon.

Components of bitter melon decrease levels of Cyclin B1 and Cyclin-D1 while elevating p21 levels, which induces cell cycle arrest. In addition, bitter melon components activate both the caspase-8 and caspase-9 pathways, in turn activating caspase-3 and PARP cleavage and inducing apoptosis. Apoptosis is also induced by activation of the MAPK pathway (phosphorylation of p38, JNK1, and ERK). Interestingly, accumulating evidence supports an increased autophagy in tumor cells exposed to some bitter melon compounds (eg., MCL and a cucurbitane-type triterpene) [23, 24]. However, since the relationships between apoptosis and autophagy are complicated, including a) autophagy limits apoptosis (cytoprotective autophagy), and b) autophagic cell death (which refers to cell death by autophagy) [25], the role of autophagy induction by bitter melon compounds in the fate of tumor cells needs to be further elucidated.