Mechanism of Action of Bioactive Compound for Cancer Prevention via NF-κB, HDACs and Autophagy Pathways

A Project Paper

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Food Science

by

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May 2019

ABSTRACT

Cancer is the major cause of death around the world that needs to be controlled and treated. Studies around bioactive compounds extracted from natural products demonstrated their great potentials as experiment targets for cancer therapies. However, the potential mechanism of action of anticancer compounds through regulating cellular signal transduction pathways and inducing cancer cell death via autophagy or apoptosis is not fully revealed. Specific targets in cell transduction pathways, NF-κB and HDACs, and autophagy pathways attract more attention in present researches around the mechanism of action bioactive compounds with anti-cancer activities. In this review, by concluding recent five years publications, it is believed that NF-κB, HDACs and autophagy pathways are essential targets for studies around bioactive compounds. Future studies may focus more on bioactive compounds that target multiple pathways to lead cell apoptosis or autophagy.

BIOGRAPHICAL SKETCH

Mengxuan Lai, graduated from China Agricultural University in 2018 with a Bachelor' s degree of Engineering. He majored in Food Science and Engineering during his undergraduate study and continue learning in Food Science as a MPS student in Cornell University. His graduate study is mainly focused on functional food, specifically, reviewing on current researches about bioactive compounds with anti-cancer activities and mechanism of action via cell signaling transduction pathways.

感谢一直陪伴我的家人和老师，以及肖娅琳女士，你们是我奋斗前进的动力。

ACKNOWLEDGMENTS

Time has always slipped away inadvertently. I have left great memories and growing better after this period of study. Thanks so much to Professor Liu's support an help during my graduate studies. Thanks to my dearest friend Yalin Xiao for her selfless support and all the people who helped in my study and life.

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INTRODUCTION

Cancer is expected to rank as the leading cause of death and the single most important barrier to increasing life expectancy in every country of the world in the 21st century. According to estimates from the World Health Organization (WHO) in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries (Bray F., et al., 2018). The International Agency for Research on Cancer provides a status report estimating the cancer incidence and mortality on the global burden of cancer worldwide in 2018. The report deduced that 8.1 million new cancer cases (17.0 million excluding nonmelanoma skin cancer) and 9.6 million cancer deaths (9.5 million excluding nonmelanoma skin cancer) arouse in 2018. In 2019, 1,762,450 new cancer cases and 606,880 cancer deaths are projected to occur in the United States. While thinking positively, the overall cancer death rate dropped continuously from 1991 to 2016 by a total of 27%, translating into approximately 2,629,200 fewer cancer deaths than would have been expected if death rates had remained at their peak (Siegel, R. L., et al., 2019).

The drop in the death rate can partially prove that people are trying to find treatments against cancer development. More and more mechanisms of tumorigenesis on cell levels have been revealed, which help researchers to develop treatments like drugs and chemotherapies that target signaling transduction pathways of tumor cells against cancer progression. Since early detection and effective treatment help increase survival rates of cancer patients (Nosrati, N., et al., 2017), prevention and treatment of cancer at early stages more promising. Due to the toxicity and damage caused by chemical synthesis products, people turn to extract functional compounds from natural products. Studies proved that fruits and vegetables can provide a wide variety of nutrients and a

range of bioactive compounds including vitamins (vitamin C, folate, and provitamin A), minerals (potassium, calcium, and magnesium), phytochemicals (flavonoids, phenolic acids, alkaloids, and carotenoids), and fibers (Liu, R. H., 2004). More and more evidence suggested that high consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases like cancer (Liu, R. H., 2013).

"Bioactive compounds" are extra-nutritional constituents that typically occur in small quantities in foods. They are proved to be valuable in the therapy of human health, for example, various studies have shown protective effects of plant-based diets on cancer (Kris-Etherton, P. M., et al., 2002). Curcumin, the main active compound from turmeric, has a wide spectrum of pharmacological activities upon multiple biological targets in preventing tumor initiation, progression, and dissemination in a number of human cancers (Zhang, P., et al., 2017). Diallyl trisulfide (DATS), a bioactive compound derived from vegetables, has been investigated as an anti-cancer and prove to arrest cancer cells at multiple stages of the cell cycle (Puccinelli, M., et al., 2017). Ursolic acid, proved to exhibit anticancer activity, anti-inflammatory effects, and induction of apoptosis in several human cancer cells, in particularly, inhibiting breast cancer proliferation by inducing cell G1/G2 arrest and regulating the expression of key proteins in signal transduction pathways, as well as inducing apoptosis in human breast cancer cells through intrinsic and extrinsic apoptotic pathways (Yin, R., et al., 2018). Various bioactive compounds appear to have benefits on anti-cancer treatments via modulating on multiple cell transduction pathways.

Recently, the increasing amount of studies around bioactive compounds and their mechanism on tumor cell signaling transduction pathways involved in regulating NF-κB, HDACs activities and modulating autophagy pathways.

NF-κB, a nuclear factor that binds to the enhancer element of the immunoglobulin kappa light-chain of activated B cells (Sen, R., et al.,1986), identified as a transcription factor family with five members, designated as p65 (RelA), RelB, c-Rel, NF-κB1 and NF-κB2 (Hoesel, B., et al., 2013). It is a key element for controlling the expression of hundreds of genes involved in immunity, inflammation, proliferation, and in defense against apoptosis (Chandrashekar, N., et al., 2012). Therefore, NF-κB is a great target for designing anti-cancer drugs, and more and more bioactive compounds in recent research are proved to regulate NF-κB transduction pathways. It is necessary to sort out commonly mechanisms of action of bioactive compounds modulating on NF-κB activities.

Autophagy, as an ATP-dependent physiological process that, together with other forms of regulated cell death, is involved in normal cytoplasmic turnover and in many important cellular functions (Levine, B., et al., 2004). It is also implicated in the rearrangement of cellular membranes, in the control of programmed cell death mechanisms, and in all those processes in which the seized material is carried, degraded, and recycled inside the lysosomes (Reggiori, F., et al., 2002). It can play a role in different types of diseases, such as cancer (Giampieri F., et al., 2019). Through its pro-survival and pro-death functions, autophagy can be related to a different stage of cancer development. Multiple studies give positive evidence to support the connection between bioactive compounds and induction of autophagy. Hence, relative intact mechanisms of action of bioactive compounds inducing autophagy are needed to be concluded as well.

Tumorgenesis is the consequences of both genetic and epigenetic alteration of the cell (Singh BN., et al., 2010). There is a rapidly emerging field that explores the link between diet and gene interactions called nutritional genomics (Vahid, F., et al., 2015). While, epigenetics is defined by the heritable changes in gene expression that does not involve alterations in the DNA sequence

(Voss, T. C., et al., 2014). Histone acetylation provides a critical mechanism for epigenetic control of gene expression (Choudhary, C., et al., 2009). The chromatin is where histone deacetylases (HDACs) deacetylate histones and alter electrostatic properties of chromatin in a manner that favors gene repression. Based on phylogenetic similarity, HDACs have been categorized into four classes, Class I (HDAC1, 2, 3, and 8); II (HDAC4, 5, 6, 7, 9, and 10); and IV (HDAC11). In recent years, studies have shown that HDACs have arisen as important therapeutic targets for various diseases (Patil M., et al, 2017). For cancer cells, uncontrolled growth can lead by deregulation of HDAC activity due to aberrant expression or recruitment to promoter regions (Singh BN., et al., 2010). So controlling the activities of HDACs is believed to be an effective mechanism of action of bioactive compounds to regulating tumor cells genesis and promotion.

This review mainly concludes recent five years of research about bioactive compounds' treatment via autophagy pathways, signaling pathways of NF-κB and histone deacetylase (HDAC) and their potential values as targets for future cancer researches around bioactive compounds.

BIOACTIVE COMPOUNDS ON INDUCING NF-κB INHIBITION

As a transcriptional factors that involved in both inflammation and cell apoptosis, NF-κB regulates a wide array of cancer-related genes, which includes survival genes like Bcl-2, TRAP, p53, and Fas 211-213; angiogenetic gene like VEGF (vascular endothelial growth factor); energy metabolism gene GLUT3; and invasive and metastatic genes like MMP (matrix metalloproteinases), ICAM-1(intercellular adhesion molecule 1, namely, CD54), ELAM-1 (endothelial-leukocyte adhesion molecule 1), and VCAM-1 (vascular cell adhesion molecule 1) (Chen, H., Liu, R. H., 2018). In most quiescent cells NF-κB dimers are bound to inhibitory molecules of the IκB family of proteins (inhibitors of NF-κB), which will further associate with the DNA-binding domains of the transcription factors thereby making them transcriptionally inactive. But It can be activated by the degradation of IκB, which leads to NF-κB be constitutively activated in many types of cancer and exert a variety of pro-tumorigenic functions. Meanwhile, NF-κB can also regulate the induction of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α). On the other hand, the addition of TNF-α stimuli can cause the activation of the inhibitors of IκB kinase (IKK), which are the major upstream regulators of NF-κB (Suh, J., et al., 2004). Once IKKs are activated and phosphorylated, they in turn cause phosphorylation and proteasomal degradation of IκBα, thus leaving the free active form of NF-κB for nuclear localization. Furthermore, studies confirmed that TNF-α can induce NF-κB's translocation from the cytoplasm into the nucleus (Yemelyanov, A., et al., 2006). As we described above, those key factors involved in the regulation of NF-κB were further demonstrated to be modulated by various bioactive compounds in recent discoveries.

6-Shogaol (6-SHO), a potent bioactive compound in ginger, is proved to inhibit TNF-a–induced phosphorylation of NF-κB in all three human prostate cancer cells (Saha, A., et al., 2014). Immunocytochemical data proved 6-SHO's ability to block the NF-κB localization into the nucleus. Also, inhibition of NF-κB was accompanied by inhibition of NF-kB downstream targets, such as cyclin D1, survivin, and cMyc. 6-SHO treatment at two doses (50 and 100 mg/kg) both inhibited the growth of HMVP2 cells without any obvious toxic reactions, which proved the potential ability of 6-SHO as a natural therapeutic agent in prostate cancer.

Lipopolysaccharide (LPS), as one of the stimuli, can trigger IκBα degradation, was found to be reduced by Longan flower and seed extracts treatment (Kunworarath, N., et al., 2016). With 1μg mL-1 of LPS treatments, the addition of Longan flower and seed extracts significantly increasing the IκBα degradation activity, which proves that phytochemicals in Logan extracts' potential on inhibited cancer through inhibition of NF-κB activation.

Pinus roxburghii essential oil (PREO), was researched on multiple cell lines, i.e. HCT-116 (colon cancer), KBM-5 (myelogenous leukemia) (Sajid A., et al, 2018). Results proved that PREO can regulate the TNF-α- induced NF-κB activation on dose-dependent to its concentration. Gene products involved in tumor cells survival, which downregulated by NF-κB activities, was also proved to be down-regulated by increasing the PREO concentration at a concentration of 100 μg/mL. Their research proved the value of PREO as anticancer, anti-inflammatory agents.

Huang and his team extracted a compound from Euphorbia sieboldiana, which found to diminish the intracellular NF-κB and translocated into the nucleus from the cytoplasm of cells for 24 h at a dosage of 10 μM (Huang, R. Z., et al., 2018). The genes downregulated by NF-κB, the mRNA levels of BCL-2, Cyclin D1, c-Myc, are all decreased by addition of this compound, from which can drive tumor cell into apoptosis.

A corresponding experiment in human malignant glioblastoma cells resulted that sulforaphane (SFN) treatment can significantly decrease NF-κB expression compared to control cells (Jiang, X., et al, 2018). It has also been proved by experiment that SFN can downregulation of the expression of antiapoptotic factors BCL-2 and BCL-xL, upregulation of proapoptotic BAX, proteolytic activation of caspase-3 (Lan, H, et al., 2017).

Former studies proved that activation of NF-κB/ p65 will leads to perturbations during the cell cycle progression and anti-apoptosis (Ku, J. M., et al, 2015). During the NF-κB signaling cascade, phosphorylation of p65 at the serine 536 positions is required for nuclear translocation (Maguire et al., 2015). Vam3, a compound derived from Vitis amurensis Rupr., was found to decrease the protein levels of TNF-α. Other than that, pretreatment with Vam3 at 2.5 μM and 5 μM reduced the phospho-NF-κB p65 expression in colon tissues (Xuan, L., et al., 2016). Similarly, for the bioactive compound linarin, proved to regulate cell cycle- and apoptosis-related signals by NF-κB/ p65 pathways. Linarin down-regulated on p65 expression in glioma cell lines in a dose-dependent manner (Zhen, Z. G., et al, 2017). Studies above further confirmed that bioactive compounds can modulate NF-κB/ p65 pathways to demonstrate their anti-proliferative and pro-apoptotic activities.

When considered the docking part for bioactive compounds on NF-κB pathways, the site of DNA-binding region (DBR) is also one direction to explore. Researchers working with 1,8- dihydroxy-4-methylanthracene-9,10-dione (DHMA), isolated from Luffa acutangala, found this compound potentially altered the binding affinity between DNA and NF-κB and led to the inhibition of NF-κB activities (Ramar, V., Pappu, S. 2016). The studies that they conducted proved that DHMA shows remarkable affinity with NF-κB p50 subunit and NF-κB- DNA by hydrogen bond interaction with active residues like Arg57, His141, Asp239 and Lys24 which are important for DNA binding to the DBR of NF-κB. Furthermore, comparing to the NF-κB -DHMA, NF-κB -

DNA-DHMA complex proved to be a more stable format. Their experiments suggest that DHMA might edit the binding abilities between DNA and NF-κB as a potent inhibitor to prevent non-small cell cancer, which adds another potential mechanism of bioactive compounds reaction with NF-κB activities in cancer chemoprevention and therapeutics.

Nowadays, more and more research around the bioactive compound is concentrated on regulating NF-κB pathways, also for its connection between anti-cancer and anti-inflammation. Recent researches stated that bioactive compounds can regulate NF-κB pathways mainly via modulating TNF-α- induced NF-κB activation and p65 activities, and downregulating on NF-κB targeted gene expressions. Further proved the importance and potential for therapy development around bioactive compounds by inducing NF-κB inhibition.

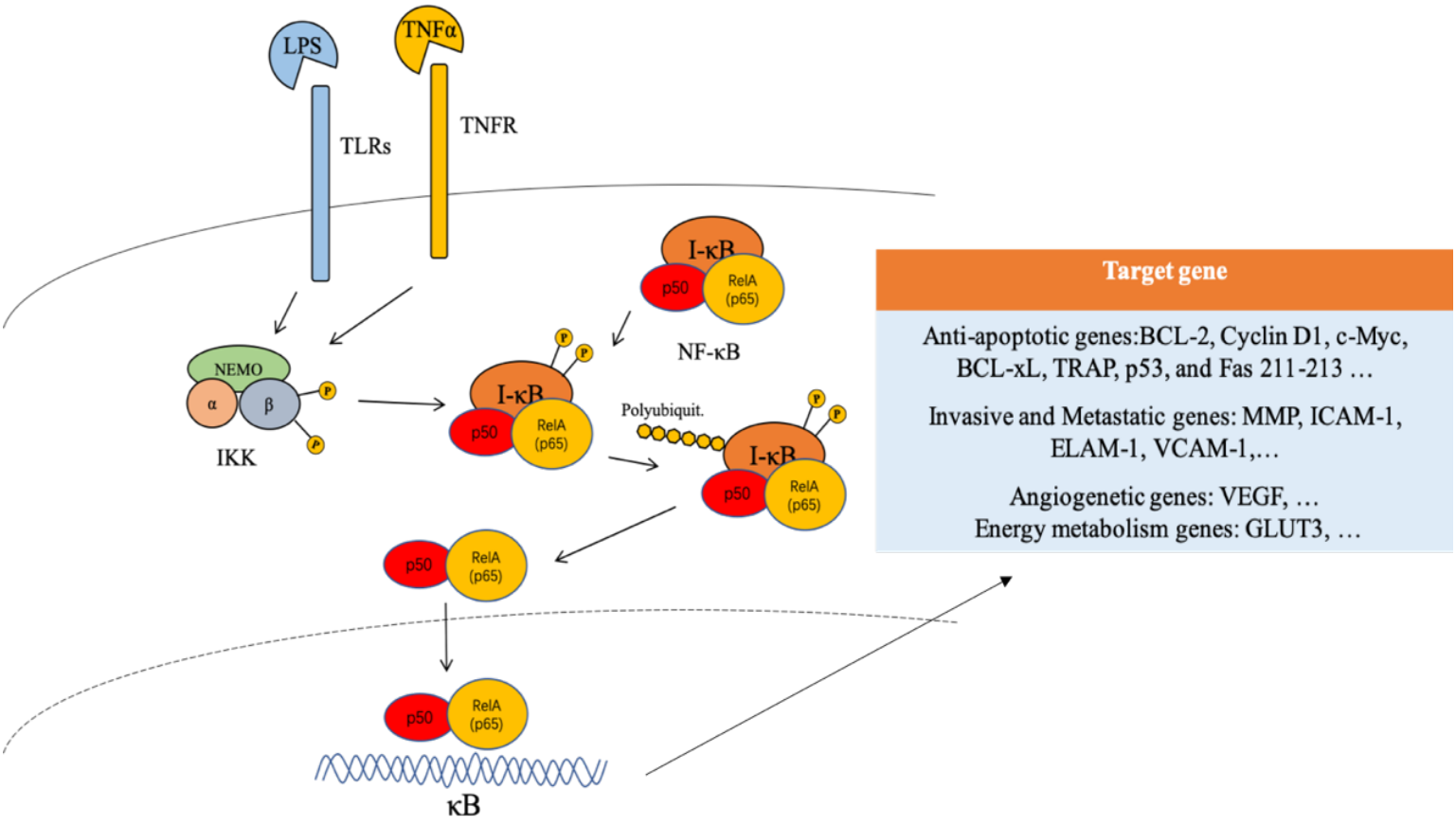

Fig. 1. Potential bioactive compounds targeted NF-κB signaling pathways

Effect of Bioactive Compounds on Autophagy Pathways

Considering different stages of cancer development, autophagy plays two different roles with pro-survival or pro-death functions. Under nutrient-limited conditions, pro-survival functions help triggering cancer cells survival. This activity of autophagy has been well established in several animal models where its suppression is effective in mitigating tumor progression in pancreatic, lung, and liver cancers (Guo, J. Y., et al., 2013; Takamura, A., et al., 2011; Yang, Z. J., et al., 2011). Key mechanisms are: under various stress conditions, including oxidative stress, DNA damage, and starvation can trigger autophagy (Shen, H. M., et al., 2011; Marino, G., et al., 2014).; as cytoprotective roles against the cytotoxic effect of cancer therapeutics can lead to autophagy induction (Wang, C., et al., 2016); autophagy increases cell motility and migration and promotes metastasis and invasion (Sharifi, M. N., et al., 2014). While if there is resistance on chemotherapies and ionizing radiation, pro-death functions can let autophagy help to kill tumor cells (Panda PK, et al., 2015). Mechanisms about its anti-cancer functions include: limits the growth of precancerous cells and mitigates cellular oxidative stress, potentially by disposing the damaged organelles, especially mitochondria (Mathew, R., et al., 2009); program cell death when massive amounts of vesicles accumulate and also maintains genome integrity so as to suppress tumorigenesis (Mizushima, N., et al., 2008; Shintani, T., et al., 2004); promotes p62 protein degradation, further preventing degradation of autophagosome and its pro-tumorigenic activity (Bjørkøy, G., et al., 2005). Multiple studies give positive evidence to support the connection between bioactive compounds and induction of autophagy.

Studies prove that bioactive compound can lead cell death via inhibiting autophagic flux and accumulating autophagosome (Lin H. et al., 2018). Physakengose G (PG), a new compound first isolated from Physalis alkekengi var. franchetii, is found to increase the intra-lysosomal pH which

leads to acidification of lysosomes and further impacts hydrolytic enzyme activity and lysosome degradation ability (Liu, et al., 2017). Researchers verified that through the impaired degradation of p62 by PG. And further evidence showed that PG-induced LAMP1 accumulation, which suggested that PG abolished lysosome acidification and lysosomal membrane permeabilization as a way to aggravated cell death.

A similar response was recorded in another research around Withaferin A (WFA), a bioactive compound derived from Withania somnifera, is proved to effectively lead to autophagosome formation during Hepatocellular carcinoma (HCC) inhibition (Siddharth, S., et al., 2019). Autophagy-related proteins accumulated, especially increased levels of LC3B-II upon drug treatment, indicated increased synthesis of autophagosomes with lysosomes to form autophagolysosomes after WFA treatment. Uniquely, in this experiment, the autophagic response was measured as well, based on the Cathepsin-D expression and activity in HCC cells. Cathepsin-D is one of the major lysosomal enzyme required for degrading cargo in autolysosomes to amino acids, fueling the metabolic reactions or repair processes in the cell (Galluzzi, L., et al., 2017), which can be an indicator as a complete autophagic response.

Interestingly, experiments have demonstrated that autophagy pathway can also regulate other cellular signaling pathways to induce cell death and apoptosis. Recent studies have found that curcumin can induce autophagy in cancer cells (Shinojima N, et al., 2007). Curcumin treatment activated autophagy by increasing protein expression of autophagy marker LC3-II, the ratio of LC3-II to LC3-I, and formation of GFP-LC3 puncta, which further demonstrates that LC3 is recruited to the autophagosomal membrane during autophagosome formation. On the other hand, curcumin treatment can also significantly increased Akt ubiquitination and Akt expression in cell insoluble fractionation at an early time-point. Evidence from a relatively early time in their

experiment supported that it is possible that Akt is degraded by an autophagy-dependent ubiquitination pathway (Guan F., et al, 2016). Their observation leads to a new notion that curcumin induces autophagy-dependent Akt degradation leading to suppression of cell proliferation and migration.

This regulation of autophagy was also found and further confirmed in another research about Δ9-tetrahydrocannabinol (THC, the main active component of marijuana, a compound that triggers autophagy-mediated cancer cell death) in glioma cell (Hernández-Tiedra, S., et al., 2016). The cannabinoid receptor 1 (CNR1) and cannabinoid receptor 2 (CNR2) are found to bind with THC which activates the ceramide synthesis. This may result in lowering the ratio of ceramide to dihydroceramide (Cer:dhCer) in the ER which can further transmit to autophagosomes and autolysosomes. The ratio changes lead to the induction of ER stress response that causes a TRIB3-dependent inhibition of the Akt-mTORC1 axis and the subsequent induction of autophagy (Salazar, M., et al., 2013). Meanwhile, lysosomal membrane permeabilization (LMP) will be activated which leads to cathepsin release and activation of apoptosis and cell death.

Walsuronoid B, a limonoid compound extracted from Walsura robusta, is found to not only initiation cell death through the mitochondria apoptotic pathway, but also lead to lysosomal membrane permeabilization (LMP) and the activation of lysosomal proteases cathepsin B and cathepsin D, triggering the lysosomal apoptotic pathway (Zhang, C., et al., 2017). Lysosomal apoptotic cell death is controlling via the ROS/p53 signaling pathways. When reactive oxygen species (ROS) occurrence, LMP is directly started due to ROS has been demonstrated to disrupt lysosomal membrane integrity (Johansson A.C., et al., 2010). ROS can trigger p53 activity, which also transcriptionally activates the lysosomal proteases cathepsins (Kruiswijk F., et al., 2015).

ROS are known to play important roles in various type of cell death, including autophagy (Xu J., et al., 2017). In research about marine-derived bioactive compounds, extracts derived from Agelas sponges were tested on HCC cells. The accumulation of ROS after the bioactive compound treatment is observed (Choi, C., et al., 2018). Evidence showed that ROS cause the increasing of ER stress and leads to autophagy in HCC cells. Their research proved that Agelas extracts may sensitize HCC cells to IR via ROS overproduction in vitro to further shift the balance in tumor cell from survival to death. On the other hand, Pei Yu and their research team found that Physagulide P (PP), a new natural compound isolated from Physalis angulate L., induce autophagy in breast cancer cells (Yu, P., et al., 2017). Further experiments discovered that is due to the generation of reactive oxygen species (ROS) and resulted in c-Jun N-terminal kinases (JNK) activation. Thereby, JNK siRNA significantly attenuated PP-triggered autophagy, and ROS scavengers almost completely reverse this apoptosis and autophagy. Their results proved the anti-cancer ability of PP and further demonstrated the potential of ROS-mediated modulation of autophagy.

Other than those signaling pathways, the PI3K/Akt/mTOR pathway is another primarily discussed pathway for bioactive compounds regulating autophagy. Harmine and harmol, belonging to the unsaturated β- carboline alkaloids, proved to exhibit stronger autophagy induction activity among other alkaloids (Cui, G., et al., 2019). Autophagy process began with autophagosomes formation, then by fusing with the lysosome, the resulting autolysosome begins to degrade the contents. This process mediated by various of autophagy- and autophagy-related proteins. Atg8 is one of the key factors in the formation of the autophagic membrane, currently as the most widely used autophagosome marker (Mizushima, N., et al., 2010), increased sharply after harmine treatment, indicating the appearance of autophagy. Atg13 and Atg101, both autophagy-related genes, also increased activities after induction of harmine. They interact with UNC-51-like kinase 1 (ULK1)

to form a complex, which further couples to the negative autophagy regulator, mTOR complex 1, and initiates autophagy (Marino, G., et al., 2014). This reveals a part of a complex signaling network involved with bioactive-compounds-induced autophagy. As in research about licochalcone A (LicA), not only PI3K/Akt/mTOR pathways related proteins were checked, but also more autophagic genes like Beclin-1, Atg5, Atg7, Atg12 which responsible for autophagosome biogenesis are tested (Tsai, J. P., et al., 2015). LicA treatment increases the expression levels of these related genes in a time-dependent manner in SiHa cells. Their research about LicA further demonstrate the complicated mechanism of autophagy and needs to be further explored. Therefore, Same pathways were further revealed in research about peiminine, a natural bioactive compound extracted from the traditional Chinese medicine Fritillaria thunbergii (Zhao, B., et al., 2018). However, researchers found that, during peiminine treatment, the main function of Akt and AMPK/ULK1 pathway was used to block autophagic flux in Glioblastoma multiforme (GBM) cells since autophagy can facilitate cancer survival under metabolic stress or induce autophagic tumor cell death. Studies about Litchi chinensis Sonnerat extracts shows identical results which a remarkable increase in the kinase ULK1 when the cell was treated extracts (Emanuele, S., et al., 2018). Further evidence showed that light chain3 (LC3), considered as a marker of autophagosome presence, was increasing after litchi extracts treatment. A significant progressive increase in the autophagic LC3-II form was also observed within 48h. Those results further tested the effectiveness of bioactive compound inducing autophagy to tumor cells.

Furthermore, a study conducted both in vivo and in vitro experiment on an aqueous extract of Allspice (AAE) showed consistent results (Zhang, L., et al., 2015). Their in vivo experiment proved that AAE greatly inhibited AKT phosphorylation as well as mTOR phosphorylation, which further proved to explain the autophagy induction by AAE. Surprisingly, they found that ULK1

activation was only found in AAE treated MCF7 cells, but not in MB231 cells. They deduced that this might be because of the activation of ULK1 results from changes in different phosphorylation sites. Furthermore, Ser757, Ser317, and Ser777 of ULK1 are also found to be the direct phosphorylation sites for AMPK in their study. The mechanism of antitumor activity conducted in their project shows great correlation with the results of their in vitro studies and was attributed to autophagy, without any detectable evidence of apoptosis.

Former researches found autophagy can be triggered under cancer cell starvation, positively regulating through the ERK1/2 pathway. So present research about mechanisms of glycyrrhizin (GL), a bioactive compound of licorice, was further digging into that direction (Zhang, X., et al., 2017). First of all, they proved that GL provoked excessive autophagy in HCC cells. Secondly, they further proved that GL can silence the Akt/ mTOR pathway. Since the phosphorylated ERK1/2 in HCC cells, it indicated that bioactive compounds can enhance the ERK1/2 activity in the induction of excess autophagic cell death pairing with the regulation on PI3K/Akt/mTOR pathways.

Since autophagy can prevent cells from undergoing apoptosis by removing damaged or superfluous proteins and organelles, it has been reported that therapies combined with autophagy inhibitors to inhibit tumor growth. Studies about WFA showed that WFA-induced autophagy in HCC cells is cytoprotective at the functional level as inhibition of autophagy using 3MA (inhibits autophagy by blocking autophagosome formation via class III PI3K inhibition), bafilomycin (inhibits autophagy by inhibiting the fusion of autophagosomes and lysosomes) or chloroquine (inhibits autophagy by elevating lysosomal pH) enhanced the efficacy of WFA treatment. Their combination index (CI) analysis showed that WFA and autophagy inhibitors synergistically inhibited the growth of HCC cells which indicating a potential combination therapeutic approach

involving WFA and clinically viable autophagy inhibitors. Similar results also conducted in the AAE research. They wider the selective range of drugs for combined therapy, by using a Rapamycin, which the main mechanism is inhibition of mTOR even at very low dose. It has been shown to be both chemo-preventive and anti-senescence compound when taken daily at a low, non-immune compromising dose (Blagosklonny, M. V., 2013). A combination was proposed by the authors, which taking AAE and rapamycin daily may contribute to a novel therapeutic avenue for the prevention of cancer. However, more evidence like a defined composition of those bioactive compounds should be provided and measured used after experiments. It will still be a challenge to give any definitive suggestion for their human use for now.

In short, it is an efficient way to control tumor cell growth via autophagy pathways. Currently, multiple studies put their attention on bioactive compounds with autophagy inducing abilities. Bioactive compounds can trigger autophagy pathways through promoting the formation of autophagosome and modulating other signal transduction pathways, such as PI3K/Akt/mTOR pathway, to initiate autophagy. Since autophagy is essential to balance the cell survive and death, the possible influence of bioactive compounds, which target autophagy pathways, on cancer therapy will be dramatic.

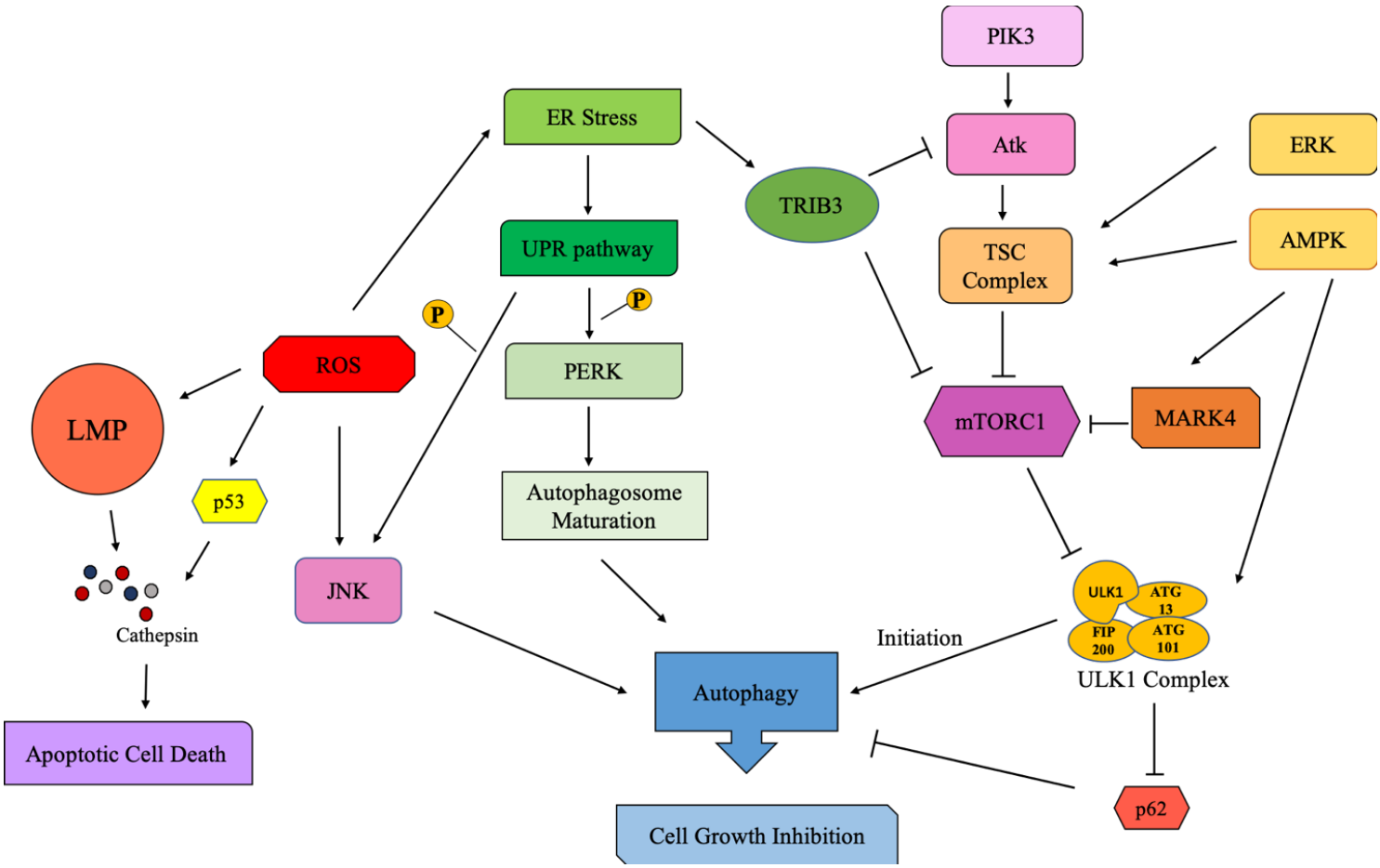

Fig. 2. Potential bioactive compounds targeted autophagy pathways

EFFECT OF BIOACTIVE COMPOUNDS ON HISTONE DEACETYLASE (HDAC)

Tumor development and progression is the consequence of genetic as well as epigenetic alterations of the cell (Mrakovcic, M., et al., 2018). Main function of histone acetyltransferases (HATs) and deacetylases (HDACs) is regulating the modification of both histone and non-histone proteins. Cancer appears under derailed acetylation-mediated gene expression, which caused by imbalanced HDAC expressions. This effect can be reversed through histone deacetylase inhibitors (HDACi) treatment via induction of cell cycle arrest, the inhibition of angiogenesis, immunomodulatory responses, the inhibition of stress responses, increased generation of oxidative stress, activation of apoptosis, autophagy eliciting cell death, and even the regulation of non-coding RNA expression in malignant tumor cells (Mrakovcic, M., et al, 2018).

Recently, more researchers paid more attention to diet and dietary bioactive compounds as a regulator of HDACs and as potential anti-cancer substances. A newly-identified flavone, from M. bontioides, has extracted bioactive compounds which downregulated the expression of histone deacetylase 2 (HDAC2) and HDAC4, leading to increased histone H3 acetylation and p21 upregulation which will further regulate on cell proliferation to control cell death (Weng J. R., et al, 2017). Inhibition of HDAC leads to protein expressions of different histone acetyltransferases (HAT) such as CBP, GCN5L2, PCAF, and p300, which found in a cucurbitacin B (CuB), a single bioactive triterpenoid compound treatment (Shukla S., et al, 2015). HDACs, especially HDAC1, HDAC5, and HDAC6 were found to be considerably downregulated by CuB starting with 60 nmol/L concentrations in H1299 cells. Results show that HDACs inhibition also helps the alternation of the accessibility of various transcriptional factors to the promoters of the tumor suppressor genes (TSG) in H1299 cells.

Similarly, outcome found in a recent paper focusing on Antrodia cinnamomea (AC) and its anti-tumor abilities (Chen, Y. C., et al., 2019). The ethanol extract of artificially cultured AC (EEAC) was able to modulate epigenetics through the inhibition of HDACs activity mediated histone acetylation. Specifically, it inhibited HDACs expression including all HDAC1 (56%), HDAC2 (29%), HDAC3 (36%) and HDAC4 (48%) with the use of 50 μg/mL. Their results further proved that EEAC helps triggering the tumor inhibition via modulating the acetylation and inhibition of histone deacetylase.

Research conducting around SFN recently also pointed out a possible way, about bioactive compounds regulating HDAC so as to control tumor cell growth (Martin, S. L., et al, 2018). hTERT, the catalytic subunit of telomerase, is found to modulated by the HDAC inhibitor SFN. Due to SFN's HDAC inhibition, it facilitates hyperacetylation and promoting an open chromatin formation, allowing known repressor proteins to interact with the regulatory region to regulate the expression of hTERT.

Another nuclear protein, Methylated CpG binding protein 2 (MeCP2), is involved in histone modification-related transcriptional repression of specific genes as well (Hansen, J. C., et al, 2010). Studies found that curcumin would cause the methylation status of MeCP2 to change in LNCaP cells (Shu, L., et al., 2011). Their results confirmed that an inhibited transcription repression can be induced by curcumin through decreased binding of MeCP2 to the methylated CpG promoter of Neurog1, which showed higher level in a high-grade tumor (Fiegl, H., et al., 2008). MeCP2 can help HDAC recruited to gene promoter during the process as well, which leads to chromatin conformational change and transcription repression of tumor-suppression genes (Singh, B. N., et al., 2010). Meanwhile, the total HDAC activities were also observed with significantly decrease which further proved the HDAC inhibitory ability of curcumin treatment.

The mechanism of bioactive compounds inducing apoptosis and mediating other tumor cell abilities through HDAC inhibition and activation of hyperacetylation is tested in the study about plant isoquinoline alkaloid berberine (BBR) treatments in lung cancer cell line (Kalaiarasi, A., et al., 2016). According to their evidence, BBR downregulates HDACs by high binding affinity against the HDAC family. BBR fits perfectly into the active site region of HDACs, and the ligands formed more hydrogen- bond interactions. Western blot revealed that the altered expression of class I HDACs and concomitant increase in acetylated histones H3 and H4 upon BBR treatment. Combining with other results from this study, through epigenetic modifications by downregulation of HDAC enzymes with histone hyperacetylation, BBR regulated sub-G0/G1 cell cycle arrest, activates the Bcl-2/Bax family to release of cytochrome c for caspase-mediated apoptosis activation and eventually suppresses the invasion and metastasis by hyperacetylation.

All those evidence supported that regulating HDACs can be a promising target for future cancer therapeutics with bioactive compounds. Table 1 concluded several pieces of research' results on recent years, which can further strengthen the point that bioactive compound with HDAC inhibiting abilities can potentially be accompanied with other mechanisms on modulating tumor cell development, which can be a significant target for future therapy design.

Table 1. Examples of Anti-Cancer Effects of Bioactive Compounds via HDACs and Autophagy pathways

Bioactive compounds	Model	Dose	Signaling pathway	Induced cell death
sulforaphane (SFN)	HCT 116 and RKO CRC cells	10.0μM	HDAC1 inhibition	Apoptosis
withaferin A (WA) sulforaphane (SFN)	Human MCF-7 breast cancer cell	1.0 μM WA and 5.0 μM SFN	HDAC1 inhibition	Apoptosis
Sulforaphane-N-acetyl-cysteine (SFN-NAC)	Glioma cell lines (U87MG and U373MG)	30μM	ERK1/2	Autophagy
cucurbitacin B (CuB)	Non–small cell lung cancer (NSCLC) H1299 cell	IC_{50} 60 nmol/L	HDAC1,HDAC5, HDAC6 inbihition	Apoptosis
	Gastric cancer cell (SGC 7901)	100nM	CIP2A/PP2A/mTORC1	Autophagy and Apoptosis
ethanol extract of Antrodia camphorata (EEAC)	HL 60 cells	/	HDAC1 inhibition	Apoptosis
	T47D breast cancer cells	25 and 50μg/mL	AKT/FOXO1	Autophagy
Curcumin (CUR)	Human prostate LNCaP cells	5μM	Total HDAC inhibition	Apoptosis
	MDA-MB-231 breast cancer cells	25μM	AMPK	Autophagy

SUMMARY AND FUTURE PERSPECTIVES

Based on our review, NF-κB, HDACs, and autophagy pathways displayed their significance in cancer treatments and their potential usage in future therapeutics. NF-κB, as a crucial target in anti-inflammation activities, also found to be valuable in cell apoptotic and autophagy. TNF-α, p65, and NF-κB downregulated genes were commonly proved to be regulated after bioactive compound treatments. As for autophagy, bioactive compounds usually initiate autophagy by leading the autophagosome formation and regulate cellular signal transduction pathways, like PI3K/Akt/mTOR and ERK pathways. Bioactive compounds promote the tumor cell from survival to death phase through autophagy. Regulation of HDACs activities may be a very promising target for future cancer therapeutics with bioactive compounds since more and more evidence supported in recent years' reports. As part of the epigenetic regulatory system, histone acetyltransferases (HATs) and deacetylases (HDACs) drive the modification of histone as well as non-histone proteins (Mrakovcic, M., et al, 2018). This gives us a possibility of cross-talk between NF-κB, HDACs, and autophagy.

As a matter of fact, studies have found the cross talk between HDAC and NF-κB. SAHA and MS-275, two types of HDACi, is proved to inducting of NF-κB target genes by NF-κB RELA/p65 hyperacetylation in combination therapy. They were found to activate autophagy and suppress the innate immune system in vesicular stomatitis virus oncolysis (Shulak, L., et al, 2014). Similar combination treatment, H40, a novel sulfur-containing hydroxamate, with SAHA triggered all differentiation, cell cycle arrest, and autophagy by hyperacetylation of histone H3 and p21CIP/WAF1 expression (Long, J., et al, 2009). Epigallocatechin-3-gallate (EGCG), proved to modulate inflammatory pathways regulated through nuclear factor-kappa B (NF-κB) signaling. Recent research observed that when EGCG was added to the cells, it reduced the binding between

p65 and p300 to the promoter region of NF-κB-regulated genes with increased recruitment of HDAC1/2 (Liu, D., et al., 2016). They found the importance of the balance between HATs and HDACs in the NF-κB-mediated inflammatory signaling pathway. However, there is not enough experimental evidence to strengthen the connection between HDACs and NF-κB and more future studies are necessary. Apart from that, according to a recent review, HDAC inhibition can also lead to autophagy via specific pathways. mTOR proved to be a major suppressive regulator of autophagy that phosphorylates which inactivates the ULK1 complex, an upstream component of the autophagic signaling machinery (Gammoh, N., et al, 2012). The research found that mTOR can be inactivated by HDAC inhibitors, therefore restores the function of the ULK1 complex and induces autophagy. Meanwhile, HDACi is also discovered to induce autophagy. On a study around EEAC, it showed interesting results connecting HDAC with autophagy. Because their results proved that EEAC can induced ER stress through IRE1 activation, which regulated anticancer protein CHOP expression as well as HDACs inhibition mediated acetyl-histones H3 and H4 activation, further leading to cell cycle arrest at G1 phase and autophagy induction in the end.

Therefore, with deeper research around signaling pathways targeting tumor cell death, bioactive compounds gain more possibilities for clinical treatment. Currently, abilities to regulate pathways in tumor cells like NF-κB, HDAC for bioactive compounds proved to be effective from many perspectives. Due to the possible connection between NF-κB, HDAC and autophagy induction, future study may dig more into multiple-channel targeted approaches to lead cell apoptosis or autophagy. Meanwhile, it is worth noticing that combination therapies gain more effectiveness during recent studies. Treatment with low concentrations of combinatorial withaferin A (WA) and SFN proved to promote cancer cell death and regulate key epigenetic modifiers in human breast cancer cells via cell proliferation and HDAC inhibition (Royston, K., et al, 2017). Hence, as more

mechanism of actions of bioactive compounds being revealed in future studies, it is promising to have less toxic and more efficient approaches in cancer chemoprevention and therapeutics.

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