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# Natural Compounds and Their Role in Autophagic Cell Signaling Pathways

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Additional information is available at the end of the chapter

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## 1. Introduction

The term autophagy (from the Greek, *auto* – oneself, *phagy* - to eat or autophagy-self eating) was first coined for structures that were observed under an electron microscope and that were consisted of single-or double-membrane lysosomal-derived vesicles containing cytoplasmic particles including organelles during various stages of disintegration [1,2]. Autophagic cell death or autophagy is a type of cell death that occurs in the absence of chromatin condensation but is associated with the massive autophagic vacuolization of the cytoplasm [3]. Autophagy is the process by which cells recycle their own nonessential, redundant, and damaged organelles and macromolecular components. Autophagy also plays its role in the suppression of tumor growth, deletion of toxic misfolded proteins, elimination of intracellular microorganisms, and pathogenesis of several diseases such as cancer and muscular disorders [4-6].

Like apoptosis, autophagy is an evolutionary conserved process that occurs in all eukaryotic cells [7]. Autophagy can be triggered as a result of nutrient deprivation, differentiation, and developmental factors. In contrast to the apoptosis, cells that die with an autophagic morphology have little or no association with phagocytes. Excessive autophagy may be attributed to crumple the cellular functions and induce cell death directly. On the other hand, autophagy can lead to the execution of apoptotic or necrotic cell death programs. It has also been suggested that autophagy may occur as a process alongside apoptosis or it may play a supportive role in apoptosis [8]. Autophagy and apoptosis differ in morphological characteristics. The causative relationship between these two has not been elucidated yet. The increase in autophagosomes indicates an increase in autophagic activity or decreased autophagosome-lysosome fusion. The most important characteristic of autophagic cell death is the appearance of double- or multiple-membrane vesicles (autophagosomes) in cytoplasm, which sequesters cytoplasmic components and organelles such as mitochondria and endoplasmic reticulum [9].

### 1.1. Role of autophagy in cancer

Cancer is an umbrella term covering a plethora of conditions characterized by unscheduled and uncontrolled cellular proliferation [10]. Cancer is the second leading cause of mortality with an incident rate of about 2.6 million cases reported annually across Europe and USA [11]. Autophagy has a multifaceted role in cancer [12,13]. Even though, present studies are only associative, autophagy most probably functions to curtail neoplasia [14]. There are several oncogenes including PI3K and Akt family members, MTOR, and Bcl2 restrain autophagy, while tumor suppressors such as PTEN, HIF1A, and TSC2 endorse autophagy [15]. Paradoxically, autophagy is double-edged sword having a role in promoting both cell survival and cell death [16]. The role of autophagy in the demise of a cell is contentious [17]. Despite the fact that number of autophagosomes increases in some dying cells, it is still ambiguous whether these structures are involved or just facilitate cell death [18]. The genetic deletion of key autophagic genes pick up the pace rather than to inhibit cell death, which accentuate the predominant survival role of autophagy [19].

Although, apoptosis and autophagy are markedly different processes but several lines of evidence have portrayed interplay between these two processes; such as the proteins from the Bcl2 regulate both autophagic and apoptotic machinery [17]. Furthermore, three types of interplay exist between autophagic and apoptotic pathways. Both apoptosis and autophagy function as a collaborator to induce cell death; autophagy act as anagonistic to hamper apoptotic cell death by promoting cell survival, autophagy act as enabler of apoptosis, and contributing in certain morphological and cellular events that occur during apoptotic cell death without leading to death in itself [8].

#### 1.1.1. Autophagy in tumor suppression

Cancer is considered as a complex group of genetic disorder with multiple causes. It is thought to be involved in perturbation of several different pathways that control and regulate the cell differentiation, cell proliferation, and cell survival. Another enigma has been the role of autophagy in tumor suppression; cancer may be protected by macroautophagy by sequestering damaged organelles, permitting cellular differentiation, increasing protein catabolism, and promoting autophagic cell death [20]. There are some experimental evidences which support the possibility that autophagy promotes the survival of nutrient-starved tumor cells and in turn contribute to cancer. Recent advances give deep insight into the molecular mechanism of autophagy. These findings more likely favor the concept that autophagy and defects in autophagy contribute to tumor suppression and oncogenesis respectively. Biochemical studies and genetic evidences designed in mammalian cells and in *C. elegans* respectively suggest that autophagy is positively regulated by the PTEN tumor suppressor gene and negatively regulated by the oncogenic Class I phosphatidylinositol 3-kinase signaling pathway. Furthermore Beclin 1, the mammalian APG gene has tumor suppressor activity and maps a tumor susceptibility locus, which is commonly deleted in human breast and ovarian cancers. The molecular mechanism of oncogenesis in human cancer can be fairly understood through genetic disruption of autophagy control. Such insights may foster the development of novel approaches to restore autophagy in the chemoprevention or treatment of human malignancies [21].

Autophagy is a kind of homeostatic mechanisms which accelerates and induces tumorigenesis when it disrupts. It removes the damaged organelles/proteins, limiting cell growth and causes genomic instability which are involved in the tumor suppression mechanism [22]. The experimental studies show that Beclin 1 is a haploin sufficient tumor suppressor gene. As this protein is used for autophagy induction and Beclin 1<sup>+/-</sup> mice were shown to be tumor prone [23]. The excessive stimulation of autophagy due to Beclin 1 protein overexpression can inhibit tumor development [24]. The accumulation of p62/SQSTM 1 protein aggregates, damaged mitochondria, and misfolded proteins due to the formation of molecular link between defective autophagy and tumorigenesis generate the reactive oxygen species (ROS) and genomic instability is observed due to the damage of DNA. ROS and the DNA damage can be prevented by knockdown of p62/SQSTM 1 in autophagy-defective cells [22]. The relationship between defective autophagy and p62/SQSTM 1 accumulation with tumorigenesis is further evidenced from a study involving p62/SQSTM 1<sup>-/-</sup> mice protected from *Ras*-induced lung carcinoma compared with wild-type animals [25]. It is further concluded that autophagy may also provide protection against tumorigenesis by limiting necrosis and chronic inflammation, which are associated with the release of pro-inflammatory HMGB1 [26]. All above findings give a concentric remark about the role of the autophagy as a mechanism of tumor suppression.

#### 1.1.2. Autophagy in tumor cell survival

The autophagy plays a predominant role in cancer cells to confer stress tolerance, which serves to maintain tumor cell survival [20]. The induction of cell death mainly relates to the knock-down of essential autophagy genes in tumor cells [27]. Cancer cells have high metabolic demands. The exposure of increased cellular proliferation and *in vivo* models to metabolic stress was shown to impair the survival of autophagy-deficient cells with compared to autophagy-proficient cells [22]. Moreover, cytotoxic and metabolic stresses, including hypoxia and nutrient deprivation, can activate autophagy for recycling of ATP and in maintaining the cellular biosynthesis and survival. Autophagy is mainly considered to be induced in hypoxic tumor cells from regions that are distal to blood vessels and HIF-1 $\alpha$ -dependent and -independent activation have been described [28]. The expression of angiogenic factors, such as vascular endothelial growth factor, platelet-derived growth factor, and nitric oxide synthase are HIF-1 $\alpha$  [28]. Human pancreatic cancer cell lines have increased basal levels of autophagy. These enable tumor cell growth by maintaining cellular energy production. Autophagic inhibition may lead to tumor regression and extended survival in pancreatic cancer xenografts [29]. In the survived cancer cells autophagy generates a state of dormancy in residual cancer cells that may further contribute to tumor recurrence and progression [30]. The increased efficacy of anticancer drugs, in response to inhibition of autophagy, supports cytoprotective role of autophagy in cancer cells. The research data indicate that *H-ras* or *K-ras* bearing activating mutations show high basal levels of autophagy in human cell lines irrespective of abundant nutrients [31]. The cell growth in these cell lines was associated with autophagic proteins. In conclusion, it is the autophagy that maintains tumor cell survival. Moreover it suggests that by blocking autophagy in tumors is an effective treatment approach [21].

## 1.2. Cross-talk between different cell-death modes

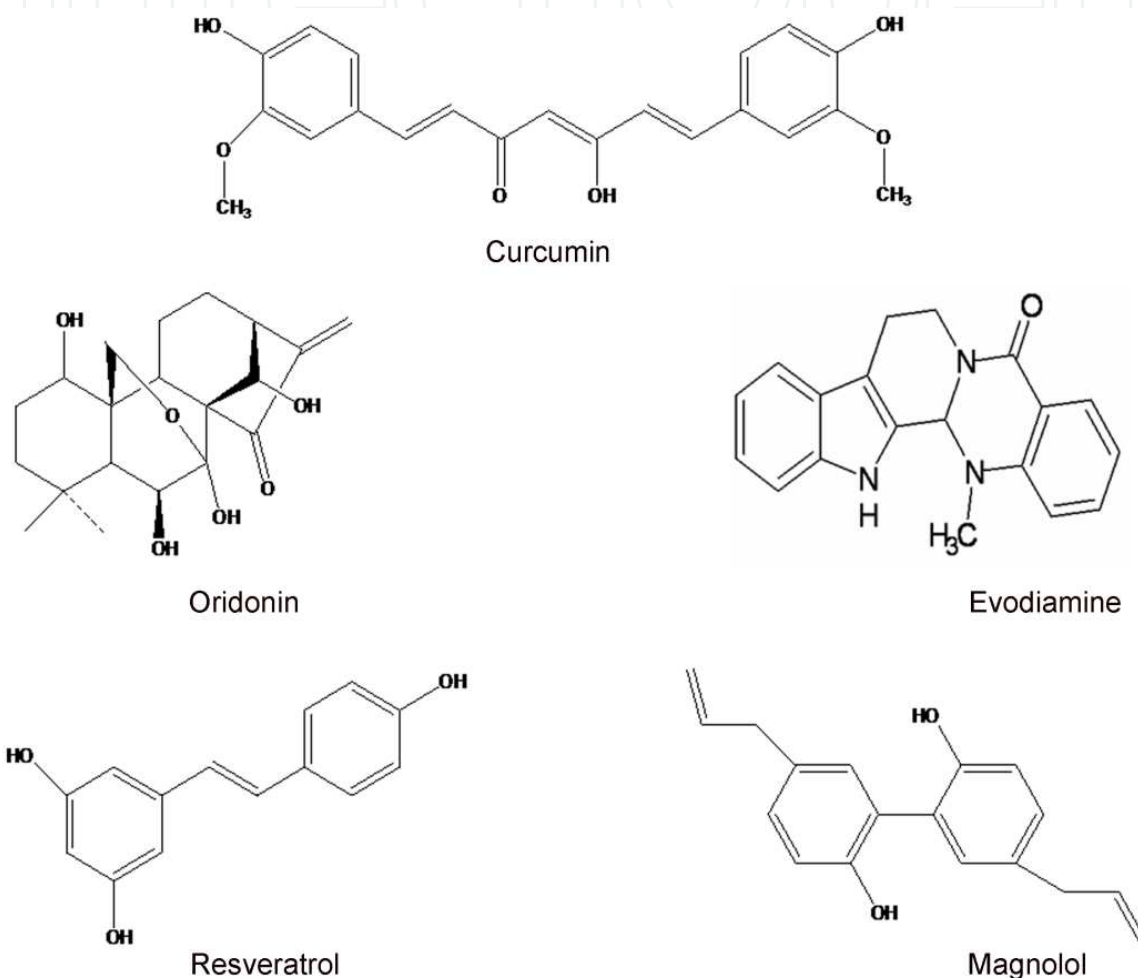
Under the physiological conditions cells show compliance with respect to how they die responding various stimuli. There are certain factors such as the type of cell, type and intensity of noxious signals, and ATP concentration that determine how cells die [19]. Acute myocardial ischemia (which is involved in the sudden fall in ATP level) induces necrosis, whereas chronic congestive heart failure (with more modest yet chronic decrease in ATP) induces apoptosis [32]. Although, a particular cell death program may preferentially be triggered in different circumstances and multiple pathways may be activated concomitantly or successively in individual dying cells [33]. Furthermore, there seems to exist an interplay among different cell death pathways. Even though apoptosis and autophagy both bear distinct morphological characteristics and physiological processes, still there exist some intricate interrelationships between them. Apoptosis and autophagy, under some conditions, play synergistic effects; while other times autophagy onsets only when apoptotic suppression occurs. Moreover, recent studies have markedly pointed out the existence of strong interconnection between apoptosis and autophagy and also strengthened the concept of simultaneous regulation of both A's that trigger cell death in cancer. The obstruction of a particular pathway of cell death may not avert the annihilation of the cell but instead may recruit an alternative path such as the broad-spectrum anti-apoptotic caspase inhibitors, zVAD-fmk, modulates the three major types of cell death. Addition of zVAD-fmk blocks apoptotic cell death, sensitizes cells to necrotic cell death, and induces autophagic cell death [34].

The overexpression of anti-apoptotic proteins may lead to the survival of injured cells where critical metabolites are provided by autophagy [35]. Nevertheless, if death stimuli persist, anti-apoptotic pathways and autophagy are unlikely to prolong and necrosis ensues [36]. Most likely, NF- $\kappa$ B, ATG5, ATP, and PARP function as molecular switches that determine whether a cell undergoes apoptosis, necrosis, or autophagy [37-39]. Protein p53 also modulates autophagy and other responses to cell stress. Recent studies reveal that basal p53 activity suppresses autophagy, whereas the activation of p53 by certain stimuli induces autophagy and the activation of p53 by different stimuli results in the PUMA- and NOXA-mediated apoptosis [40-42]. In addition, low and moderate concentrations of some agents have been revealed to induce apoptosis, but increasing the concentration of the same agent triggers necrosis. The challenge is, therefore, not only to understand the mechanisms leading to cell death but also to categorize the connection at the molecular level between different modes of the cell death.

In this chapter, we discussed the natural compounds and their mechanisms by which they induce apoptotic and autophagic cell death in cancer cells and their potential as a novel strategy for the treatment of cancer. We also presented the results of our previously published natural compounds screened against gastric cancer [43]. The screen was used to identify new targets to combat cancer or to identify selective natural compounds those target to apoptosis or autophagy signaling pathways. In this chapter, we reviewed the main effects of natural compounds on the different autophagic cell death signaling pathways. In addition, we focused on highlighting several representative plant-derived natural compounds such as curcumin, resveratrol, evodiamine, oridonin, and magnolol (structures of these compounds are shown



in Fig. 1) that may lead to cancer cell death - for regulation of some core autophagic pathways, involved in Ras-Raf signaling, Beclin-1 interactome, BCR-ABL, PI3K/Akt/mTOR, FOXO1 signaling, the NF- $\kappa$ B-mediated pathway, the PI3K/Akt signaling pathway, p53 and other main pathways. Two of the identified autophagy inducer natural compounds, magnolol and evodiamine, have been discussed in detail, while the other natural compounds, which had shown an essential role in autophagic cell signaling pathways, been reviewed recently by Zhang et al., 2012 [44].



**Figure 1.** The structure of natural compounds that act on autophagic cell signaling pathways.

## 2. Methods

The rationale for overall project design was based on assumptions as presented above, which were motivated by the set of issues. The main goal of the study was to explore naturally occurring compounds that exhibit cytotoxic activity toward cancer cells. To search for compounds with significant cytotoxic activity and unprecedented chemical structures from a

variety of traditional Chinese medicines, the crude ethanolic extracts of 300 species of herbal plants, traditionally used in China for the treatment of a variety of diseases, and four hundred TCM compounds were screened. Understanding the interplay of different cancer-related signaling pathways is important for the development of efficacious multi-targeted anticancer drugs. Hence, the underlying molecular mechanisms of the above mentioned natural compounds have been elucidated, which induced cell death.

2.1. Screening strategies

The screening strategy has been shown in schematic form in Fig. 2. Several cancer cells, including SGC-7901, U87, PANC-1 and A-375 cells were cultured in DMEM supplemented with 10% fetal bovine serum and were exposed to different Chinese medicinal herbs extracts and TCM compounds.



**Figure 2.** Schematic presentation of strategy of identification, isolation, structure elucidation, and screenings of Traditional Chinese medicines (TCM) against cancer cells.

2.2. Acridine orange staining

Acridine orange staining assay was performed according to published procedure [45]. In brief, cells were incubated without (control) and with respective compounds and with rapamycin (positive control group) for indicated time periods and then acridine orange at

a final concentration of 1 mg ml<sup>-1</sup> was added to cells for a period of 20 minutes in the dark at 37 °C. Then, cells were washed twice with PBS. Images of cells were obtained under fluorescence microscopy

## 2.4. Flow cytometric quantification of Acidic Vesicular Organelles (AVOs)

AVOs formation (autophagosomes and autolysosomes) is a characteristic feature of Autophagy [17]. Furthermore, for quantification of AVOs, we used flow cytometry after the cells were stained by Acridine Orange (AO) [46]. AO is a weak base that accumulates in acidic spaces and gives bright red fluorescence (punctate staining (dots) in the cytoplasm which is detected by fluorescence microscopy and the formation of AVOs can be quantified by flow cytometry. The intensity of the red fluorescence is proportional to the degree of acidity.

## 3. Results

The large collections of Traditional Chinese medicinal herbs and natural compounds libraries have been used to identify anticancer TCM herbs and natural compounds associated with various cancer specific cellular processes such as apoptosis or autophagy. A list of natural compounds those expressed cytotoxic activity against gastric cancer cells has been presented in Table 1 [43]. Using Traditional Chinese medicinal herbs and natural compounds libraries screen, we discovered several TCM compounds that showed potential anticancer activities [43]. In a natural compounds screen with glioma brain tumor cells, our results revealed that alantolactone and Pseudolaric acid B have shown selective anti-glioma activity with lesser toxic effect over liver and kidney [47,48]. Furthermore, we reported that Dracorhodin perchlorate regulates PI3K/Akt, p53 and NF-κB pathways that are frequently deregulated in cancer and their simultaneous targeting by Dracorhodin perchlorate could result in efficacious and selective killing of cancer cells [49]. Through the screen of natural compounds for apoptosis and autophagic cell signaling pathways, we identified several compounds including costunolide and xanthoxyletin that induce cell death via apoptotic pathways [50,51]. In addition, we also found that several compounds such as curcumin, resveratrol, evodiamine, oridonin, and magnolol induce autophagy and act on autophagic cell signaling pathways (unpublished data). Furthermore, we examined the role of evodiamine- and magnolol-induced autophagy in cancer cell death [52,53]. The role of each of the natural compound in autophagic cell signaling will be discussed later in this chapter.

## 4. Discussion

### 4.1. The role of natural compounds in autophagic cell signaling

Plants have a long recorded history to employ in the treatment of cancer [54] and represent the most important direct antecedent to contemporary anticancer drugs [55]. Recently, some of the most encouraging clinical evidences and promising anticancer natural herbal com-



pounds let us to reconstruct the story of these plants and their ultimate role in chemotherapy [56]. To provide a paradigm of the most contemporary progress in this field, there were number of compounds namely artesunate, homoharringtonine, arsenic trioxide and cantharidin isolated from natural products and have the potential for use in cancer therapy. For many years, apoptosis has taken a center stage as the most important mechanism of programmed cell death in mammalian tissues. Apoptosis is a common mode of action for chemotherapeutic agents including natural product-derived drugs [57,58]. Four categories of dynamic cellular activities, which lead to cell death, have been described: apoptosis, autophagy, necrosis, and mitotic catastrophe [59]. With contemporary development in cancer research, it has also been increasingly noted that conventional chemotherapeutic agents not only elicit apoptosis but also activate other modes of cell death such as necrosis, mitotic catastrophe, senescence, and autophagy [60].

Sr. No	English Name	Chinese Name	M.W	IC <sub>50</sub> ( μM )
1	Artesunate	青蒿琥酯	384.43	44.7±5.3
2	Isoalantolactone	异土木香内酯	232.318	34.9±3.4
3	Cucurbitacin II a	雪胆素甲（雪胆甲素）	574.702	17.9±3.4
4	Tubeimisine-1	土贝母苷甲	1319.43	20.7±1.3
5	20(S)-Ginsenoside Rh2	20(S)-人参皂苷Rh2	622.6	18.9±1.3
6	Shikonin	左旋紫草素	288.295	19.7±0.9
7	Cepharanthin	千金藤素	606.707	20.4±3.6
8	Evodiamine	吴茱萸碱	303.358	11.7±1.9
9	Chelerythrine	白屈菜红碱	348.36	18.4±2.6
10	Patchouli alcohol	百秋李醇	222.366	29.3±3.8
11	Dracorhodin perchlorate	血竭素高氯酸盐	366.75	54.9±4.3
12	Resveratrol	白藜芦醇	390	16.4±2.7
13	Podophyllotoxin	鬼臼毒素	414.405	19.4±2.7
14	Oridonin	冻凌草甲素	364.43	18.4±0.7
15	Curcumin	姜黄素	368.38	28.7±2.3
16	Magnolol	厚朴酚	266.33	64.9±4.3
17	Costunolide	木香烃内酯	232.32	37.7±3.3
18	Pseudolaric acid	土荆皮乙酸	430.491	8.7±1.9

**Table 1.** MMT assay results of the cytotoxic activities of various compounds against cancer cells with their IC<sub>50</sub> values.

Autophagy represents a major route for degradation of aggregated cellular proteins and dysfunctional organelles. Accumulated lines of evidence have recently revealed that targeting autophagic signaling pathways might be a promising avenue for potential therapeutic purposes. Alterations in autophagy are thought to play an important role in the pathogenesis of many diseases—for example, Autophagy is closely associated with tumors and plays an important role in human tumor suppression, so inducing autophagy is a potential therapeutic strategy in adjuvant chemotherapy [61,62]. Many studies have demonstrated that anticancer agents induce autophagy, leading to the implications that autophagic cell death may be a vital mechanism for tumor cell killing by these agents [63] and are beneficial in the context of various models of cancer cells. The autophagy machi-

nery interfaces many cellular stress-response pathways, and recent studies depict that defects in autophagy lead to cancer cell proliferation [64]. The regulation of autophagy in cancer cells can enhance tumor cell survival, yet can also suppress the initiation of tumor growth. Understanding the signaling pathways involved in the regulation of autophagy is crucial to the development of anticancer therapies [21]. In this chapter, we reviewed the natural compounds molecular mechanisms of autophagy and examined ongoing drug discovery strategies for modulating autophagy for therapeutic benefits. The natural anti-tumor agents have led to enhanced enthusiasm for the development of drugs that target the various aspects of the autophagic pathways. Some of these autophagic cellular approaches by representative natural compounds in autophagic induced cell death have been outlined in Fig. 3. In addition, magnolol and evodiamine have been illustrated in detail.

#### 4.2. Magnolol

In our own studies, performing the screen for natural compounds that induce autophagy, we identified magnolol [52]. Magnolol, a natural compound, has been reported to inhibit growth in a variety of tumor cells [65]. Several researchers reported that magnolol-induced cell death involve apoptosis while Li *et al* [66] reported that magnolol-induced death occurs via autophagy but not apoptosis. We observed that there was no significant formation of AVOs at low concentration while AVOs were formed at a higher concentration of magnolol treated cells. The formation of acidic vesicular organelles (AVOs) is one of the characteristic features of cells, which passes through process of autophagy after their exposure to different autophagy inducer agents [67,68]. Autophagic vacuoles (AV) or autophagosomes are formed as result of sequestering of parts of the cytoplasm or entire organelles respectively during the process of autophagy [62]. Currently autophagic cell death has been studied as a potential method for cancer therapy. To determine the role of magnolol-induced autophagy in killing the SGC-7901 cells, we added the autophagy inhibitor, 3-methyladenine (3-MA), which controlled autophagy pathway at various points [8]. In contrast to the previous report [69], it was found that magnolol-induced cell death was not suppressed when treating the cells in combination with 3-MA. These results showed that magnolol-induced autophagy is not involved in the induction of SGC-7901 cell death. In addition, the findings also demonstrated that magnolol-induced autophagy may has an effect on ATP level in the SGC-7901 cells and supported those observations which showed that autophagy may alter the morphological and cellular events (ATP, cells blebbing and DNA fragmentation) that take place in apoptotic cell death, without leading to cell death in itself [8].

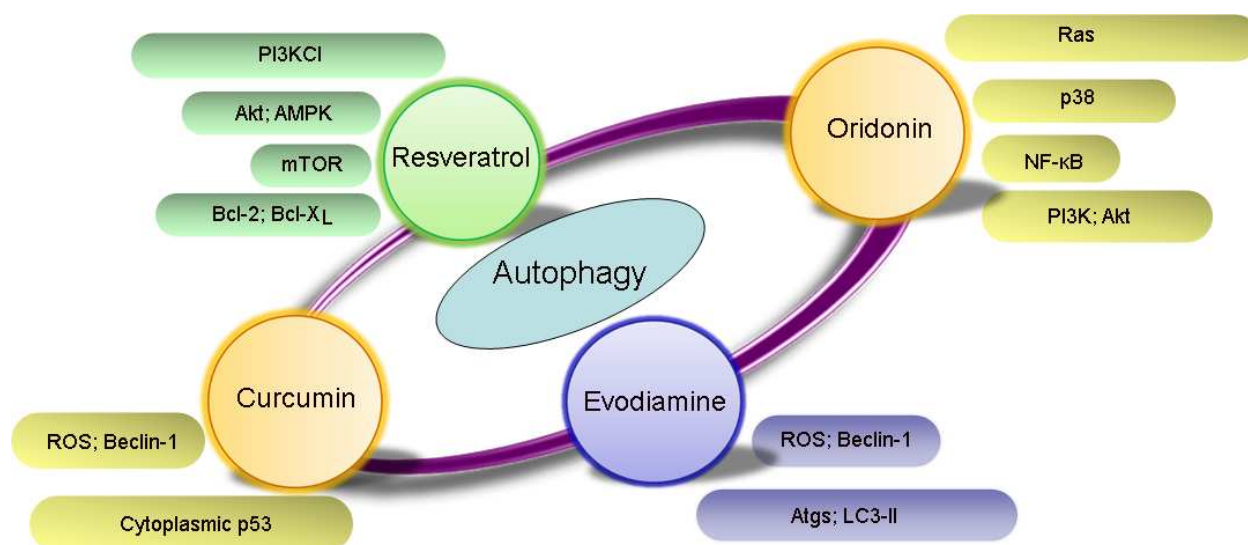
#### 4.3. Evodiamine

Evodiamine is a naturally occurring quinolone alkaloid found in the fruit of *Evodia rutaecarpa*. The data of several studies concerning the cytotoxic activity on cancer cells demonstrated that evodiamine inhibited the growth of several tumor cells [70]. Results from our screen indicate that evodiamine induced apoptosis and autophagy simultaneously in human gastric cancer cells. Evodiamine has been reported as an inducer of autophagy in human cervical carcinoma HeLa cells [71]. Autophagy is closely associated with tumors and plays an impor-

tant role in human tumor suppression, so inducing the autophagy is a potential therapeutic strategy in adjuvant chemotherapy [61,62]. When the cells are exposed to various autophagy inducer agents, they form acidic vesicular organelles, which is an important characteristic of autophagy [67,68]. Thus, we observed the effect of evodiamine treatment on the formations of AVOs in SGC-7901 cells using fluorescence microscopy after staining with the lysosomotropic agent, acridine orange (AO). These findings indicate that evodiamine, a natural compound, has the potential to activate autophagy in gastric cancer cells. This result of evodiamine is also consistent with the results of the studies reporting that natural compounds can induce autophagy in various cancer cells. We also demonstrated that evodiamine-induced cell death was partially suppressed when the cells were treated in combination with specific autophagy inhibitor, 3-MA. These results showed that evodiamine-induced autophagy was partially involved in the cell death of cancer cells [52]. While our recent studies demonstrated that autophagy inhibition enhanced evodiamine-induced apoptosis in prostate cancer cells, indicating a survival function of autophagy (unpublished data). These results corroborate the line of evidence demonstrating that evodiamine-induced autophagy, implicated in cell survival, contributes to the cytoprotective role of autophagy [71,72]. These facts demonstrated that there is still a great discrepancy between roles of natural compounds-induced autophagy in cancer cells. The role (or more likely roles because as we discuss, distinct functions for autophagy occur at different times) of natural compounds-induced autophagy in cancer, is a topic of intense debate. These responses might vary with cell type and type of stress, and will undoubtedly reflect the nature of the mutational events occurring in the tumor cells, not only that of *BECN1* and the PI3K pathway as described above, but also p53 status [73,74]. Moreover, it is believe that in short term assays, the phenomenal protection caused by autophagy inhibition may be due to delay in cell death instead of true protective effect and this inhibition causes an increase in tumor cell clonogenic growth after drug treatment. In most of the examples cited above, this appears as a starking effect, as the whole debate is about the drug induced autophagy and in response to which the cells die (or cells found dead). This novel approach emerged as an important point because a recent study also supports this myth that rapamycin-induced autophagy can protect various tumor cell lines against apoptosis induced by general apoptotic stimuli [75] and may have a similar effect on the action of anticancer agents. Moreover, similar to etoposide [74], it has been observed that knockdown of *Atg* genes does confer a clonogenic survival advantage to cells after treating with anticancer agents and the used cells have profound defects in their apoptosis machinery [73,74].

#### 4.4. Role of autophagy in cancer: Science or myth?

It is generally believed that the complex two-faced nature of autophagy in tumor cell survival versus death may help in determining cancer therapeutic potential. So inhibiting autophagy may enhance anti-cancer drugs efficacy fairly used in chemo- and radiotherapy-induced activation of autophagic signaling pathways and which may augment anti-tumor activity, and thus efficacy of radiation and/or anti-cancer drugs. We are still at the initial stages of understanding the complex interplay of autophagy and cancer, but it is incontrovertible that autophagy is deeply integrated into metabolism, stress response and cell-death pathways [64]. Preliminary evidences, in addition to some natural compounds that induced autophagic cell



**Figure 3.** Representative natural compounds (Resveratrol, Oridonin, Curcumin, and Evodiamine) targeting autophagic pathways.

death, support the idea that natural compounds-induced autophagy enhances tumor cell survival. Anticancer agents that can be involved in the induction of autophagy include tamoxifen, arsenic trioxide, rapamycin, histone deacetylase inhibitors, temozolomide, ionizing radiation [63], vitamin D analogues [76], and etoposide [74]. In addition, several natural compounds, (curcumin, resveratrol, evodiamine, oridonin, and magnolol) in our natural compounds libraries screen for autophagy inducer, were found to be involved in autophagy. However, despite the above examples, is autophagy really an important cell death mechanism? is highly controversial.

Controversy remains as whether autophagy limits or promotes tumor malignancy, till genetic inactivation of autophagy, is found to promote tumorigenesis constituting a new category of tumor suppressors including Beclin 1. Some of the oncogenes including PI3K/AKT/mTOR and Bcl-2 inhibit autophagy causing tumor cells proliferation, while the other oncogenes including Ras and myc stimulate autophagy [63]. The significance of autophagy at different stages of tumor progression can be evaluated considering these kaleidoscopic effects. Further investigations on natural compounds into the impact of autophagy inactivation are warranted. All of the above data draw many questions in autophagy mechanistic pool focusing whether autophagy is really an important mechanism of tumor cell killing by anticancer agents in cells having ability to undergo apoptosis. Rigorous examination also manifest the speculation whether bona-fide cancer drugs are actually capable of killing tumor cells via autophagy, is needed. To answer these questions is the need of the hour, as it may determine the route causes and may best streamline the contradictory approaches in developing effective combination therapies by regulating autophagy along with anticancer agents. In conclusion, we now have sound justifications to visualize that manipulation of autophagy may provide a useful way to prevent cancer development, limit tumor progression, and increase the efficacy of cancer treatments. This comprehension seems reasonable due to the fact that drugs induce autophagy, such as rapamycin (as discussed above), and is rapidly gaining a better understanding of how



this process works based on the effects of targeted inactivation of autophagy regulators in mouse models and human tumor cells. More contradictory messages come when we consider how autophagy affects the ways by which the tumor cells die when we treat them with anticancer agents. Over the last several decades the therapeutic use of natural compounds that induce autophagy has been leading us to the implications that autophagic cell death may be a vital mechanism of tumor cell killing by these agents.

## 5. Concluding remarks and future perspectives

Accumulated lines of evidence have recently revealed that targeting autophagic signaling pathways may be a promising avenue for potential therapeutic purposes. Although this chapter has focused on natural compounds and their role in autophagic cell signaling pathways, future studies investigating the mechanisms of natural compounds-induced autophagy and their role in cancer cell death. Progress towards better treatment and understanding by natural compounds may be made by further examining the role of natural compounds and crosstalk between the apoptosis and the autophagy. Despite these obstacles, many compounds bring the hope that with sufficient modification by tools of structural biology and combinatorial chemistry, it might be possible to derive sufficiently potent drugs to target core autophagy pathways, and even autophagic networks in cancer cells, rather than their individual gene or protein components. Indeed, as discussed above, the generally used cancer therapeutics, especially natural compounds abolish tumors by inducing apoptosis and autophagy. On the other hand, a better but growing setting approach is required to make a distinction between the survival-supporting and death-promoting roles of autophagy. Furthermore, for selectivity and specificity, role of autophagy, along with the elucidation of the signaling pathways those confer the autophagic response downstream of different stimuli and activate the specific and therapeutic response, is desired.. In the end we have coherent arguments in favor of principal paradigm that disease-associated autophagy could be selectively targeted for therapeutics.

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