Journal of Cancer Research and Clinical Oncology (2024) 150:474
https://doi.org/10.1007/s00432-024-05982-1

REVIEW

Epigenetic modulation of autophagy pathway by small molecules in colorectal cancer: a systematic review

Mozhdeh Zamani[1,2] · Farima Safari[1] · Morvarid Siri[1] · Somayeh Igder[3] · Niloofar khatami[1] · Sanaz Dastghaib[4] · Pooneh Mokarram[5]

Received: 24 July 2024 / Accepted: 30 September 2024

Abstract

Purpose Colorectal cancer (CRC) remains a global health challenge with limited treatment success due to drug resistance. Recent research highlights the potential of small molecules to modulate CRC by targeting epigenetics or autophagy pathways. This systematic review explores the epigenetic effect of small molecules on autophagy in CRC, aiming to identify novel therapeutic strategies.

Methods Following PRISMA guidelines, we systematically reviewed 508 studies from PubMed, Scopus, and Web of Science databases until August 13, 2023.

Results Eight studies met inclusion criteria, examining the role of small molecules as epigenetic modulators (Histone acetylation/deacetylation, DNA methylation/demethylation and gene expression regulation by miRNAs) influencing the autophagy pathway in CRC. The studies encompassed in vitro and animal model in vivo studies. Small molecules exhibited diverse effects on autophagy in CRC. For instance, panobinostat promoted autophagy leading to CRC cell death, while aspirin inhibited autophagy flux, reducing aspirin-mediated CRC cell death. The epigenetic modulation of autophagy by various small molecules differently affects their anticancer effect, which underscores the complexity of therapeutic interventions.

Conclusion Understanding the intricate dynamics among small molecules, epigenetic modifications, and autophagy in CRC is crucial for developing targeted therapeutic strategies. Considering the dual role of autophagy in tumorigenesis and tumor suppression, administration of these small molecules may differently affect the cancer cell fate and drug response or resistance based on their effect on the autophagy pathway. Therefore, recognition of the epigenetics mechanism of anticancer small molecules on autophagy may contribute to deciding how to prescribe them for better CRC treatment.

Keywords Small molecules · Colorectal cancer · Autophagy · DNA methylation · Histone acetylation · miRNA

Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide, characterized by an unfavorable prognosis and significant metastasis incidence (Rawla et al. 2019).

Various genetic and epigenetic alterations in different signaling pathways are considered to be fundamental in the development and progression of CRC (Housini et al. 2023).

Epigenetics modifications consist of hereditary processes that control gene expression without changing the DNA sequence. DNA methylation, histone modifications, and regulation of gene expression by non-coding RNAs (ncRNAs) are examples of significant epigenetic changes (Nagaraju et al. 2021). In mature organisms, critical processes such as epigenetics are necessary for the growth and specialization of cell lineages (Duncan et al. 2014).

✉ Sanaz Dastghaib
suny.respina@gmail.com

✉ Pooneh Mokarram
mokaram2@gmail.com; mokaramp@sums.ac.ir

1 Autophagy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

2 Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

3 Department of Clinical Biochemistry, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

4 Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

5 Autophagy Research Center, Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran

Published online: 23 October 2024

Aberrant epigenetic modifications are related to various diseases such as cancer. For instance, DNA promoter hypermethylation causes down-regulation of tumor suppressor genes (TSGs) such as Secreted protein acidic and rich in cysteine (SPARC) (Nagaraju and EI-Rayes 2013) and DNA promoter hypomethylation leads to up-regulation of oncogenes such as Long interspersed nuclear element-1 (LINE-1) (Szigeti et al. 2022), which contribute to the CRC tumorigenesis and progression. Different histone modifications include acetylation, methylation, phosphorylation and ubiquitylation. Among these changes, histone hyperacetylation of oncogenes and histone hypoacetylation of TSGs lead to oncogene overexpression and TSG down-regulation, respectively, which promote cancer cell growth and survival (Ghavami et al. 2022).

Prominent non-coding RNAs (ncRNAs) including, small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs) are essential to epigenetic processes (Ghavami et al. 2022). Among these ncRNAs, miRNAs play a key role in the post-transcriptional regulation of gene expression. The miRNAs bind to the target genes and inhibit their expression. According to their targets, miRNAs can act as oncogenes (oncomiRs) or tumor suppressor miRs. Overexpression of oncomiRs such as miR-21 (Far et al. 2023) and downregulation of tumor suppressor miRs such as miR-34a (Zhu et al. 2019) can promote cancer development and progression.

Epigenetic alterations in tumor suppressor genes (TSGs) or oncogenes contribute to the dysregulation of cell death signaling cascades such as apoptosis, autophagy, necrosis and ferroptosis (Yan et al. 2024).

As the principal programmed cell death cascade, apoptosis represents complex regulatory mechanisms in CRC through either intrinsic or extrinsic pathways. Ferroptosis as an iron-dependent programmed cell death and necrosis as a non-programmed cell death are also involved in the development of CRC (Liang et al. 2023).

Another alternative cell death pathway, autophagy, plays an important dual role in CRC tumor suppression or promotion. Autophagy is a highly conserved process that includes sequestering and transporting redundant cytoplasmic components to lysosomes via double-membrane autophagosomes. Autophagy is essential for preserving cellular viability and homeostasis in response to a variety of internal and external stresses (Karmacharya and Jung 2023). Through its integration with nucleus transcriptional and epigenetic control, this mechanism supports the cellular homeostasis of normal cells. Autophagy-related genes (ATGs) are primarily regulated by epigenetic processes at both the transcriptional and post-transcriptional stages. Under some circumstances, epigenetic changes of genes and autophagy regulators can either stimulate or inhibit autophagy (Hu 2019). Numerous human diseases, such as autoimmune disorders, neurological illnesses, and malignancies including CRC, are linked to autophagy dysregulation (Ichimiya et al. 2020). Impaired autophagy increases the likelihood of carcinogenesis through an increase in reactive oxygen species (ROS), damaged organelles, and cancer-causing proteins, (Debnath et al. 2023; Rakesh et al. 2022). The autophagy pathway plays a context-dependent role in CRC, either causing tumorigenesis or establishing a rescue system under adverse conditions, depending on the specific situation (Bhol et al. 2020; Darwiche 2020).

The primary clinical management of CRC involves chemotherapy, radiotherapy, surgery, and target therapy, till now. Despite recent advancements in chemotherapy, patients still cannot be cured due to resistance to conventional cytostatic drugs like 5-Fluorouracil (5-FU), platinum-based drugs (such as oxaliplatin), and irinotecan (Hammond et al. 2016). Chemotherapy resistance has emerged as a significant obstacle to the success of antitumor treatments over the years. As a result, it is unequivocally apparent that CRC treatment requires novel therapeutic approaches to combat drug resistance (Sadida et al. 2024). Considering the importance of epigenetics and death signaling pathways such as autophagy in CRC tumorigenesis and drug resistance, they have recently been considered as promising therapeutic targets in CRC treatment.

Recent research has revealed the anti-tumor properties of novel small molecule compounds, including those found in chemo-resistant cancers, such as CRC (Yang et al. 2023). Small molecules are organic compounds with low molecular weight, possessing unique chemical properties that enable them to interact with specific targets within cells (Li and Kang 2020). These compounds can modulate cell proliferation, migration, and metastasis by directly targeting tumor cells and subcellular structures or influencing the tumor microenvironment (Moshawih et al. 2022). Several small molecules have been introduced as anticancer agents by targeting epigenetic modifications (Nagaraju et al. 2017; Bajpai et al. 2024) or autophagy signaling pathway (Zhu et al. 2018; Pan et al. 2018; Zhou et al. 2024).

The utilization and progression of small molecules in the investigation of epigenetic modifications have had a profound influence on the domain of cancer research (Wang et al. 2021). Small molecules selectively target epigenetic marks and enzymes, allowing them to modulate gene expression patterns and cellular phenotypes, thereby paving new therapeutic interventions. Small molecules possess unique chemical properties and selectively target specific cellular components, providing immense potential for modulating epigenetic modifications (Xiao et al. 2021; Ghavami et al. 2022). For instance, ganetespib small molecule

inhibits the heat shock protein 90 (HSP90), which results in the downregulation of DNA methyltransferase enzymes (DNMT), decreases DNA methylation, and finally re-expression of some tumor suppressor genes including MutL protein homolog 1 (*MLH-1*), *P16* and *SPARC* (Nagaraju et al. 2017). The combination of two small molecules including regorafenib and dual Janus kinases/ Histone deacetylase inhibitor (JAK/HDACi) was more effective in reducing colorectal tumor growth and metastasis (Bajpai et al. 2024). Several small molecules also target autophagy to perform their antitumor function in CRC (Du et al. 2023). Curcumin (Zhu et al. 2018), Bufalin (Pan et al. 2018) and resveratrol (Zhou et al. 2024) are some examples of natural small molecules, which promote CRC cell death through autophagy activation.

While epigenetics and autophagy are distinct cellular processes, as a new-known action mechanism of small molecules, they may act as epigenetic modulators to regulate tumor development and therapeutic response by interacting with autophagy (Patnaik 2019; Li et al. 2022). In addition, the contribution of these small molecules to the maintenance of CRC stem cells, which sustains tumor cell viability during dormancy and ultimately contributes to tumor recurrence, demonstrates the significance of autophagy epigenetic modification in CRC (Nalli et al. 2021; Zalewski et al. 2021). Therefore, it is critical to comprehend the effects of small molecules with variable epigenetic modifications on autophagy pathways in CRC to develop novel therapeutic strategies.

In this systematic review, we delved into the effects of small molecules with dynamic epigenetic modifications on the autophagy pathway in CRC. Through elucidating the complex dynamics among small molecules, epigenetic modifications, and autophagy, our objective is to provide insight into prospective therapeutic approaches that specifically target autophagy and enhance treatment efficacy in CRC.

Materials and methods

Search strategy

The systematic review was conducted by pre-defined criteria and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We conducted a comprehensive search strategy using Medical Subject Headings (MeSH) terms to identify relevant studies from PubMed, Scopus, and Web of Science databases. The following search strings were used: ("Colorectal Cancer" OR "Colon Cancer" OR "Rectal Cancer") AND ("Autophagy" OR "Cell Death") AND ("Epigenetics" OR "Epigenomics" OR "DNA Methylation" OR "Histone Modification" OR "Histone Acetylation") AND ("Small Molecules" OR "Aspirin" OR "Panobinostat" OR "Se-allylselenocysteine" OR "Zebularine" OR "Catalpol" OR "Indicaxanthin").

We also used truncation symbols to capture variations of keywords, for example, "Autophag*" to include terms such as "Autophagy", "Autophagic" and "Autophagies".

The searches were limited to studies published from the inception of the databases to August 13, 2023, to identify studies on small molecules as epigenetic modulators involved in targeting autophagy mechanisms in colorectal cancer. We also applied filters for article types (original research studies including in vitro, in vivo and clinical studies) and languages (English only). The searches were performed independently by two authors and results were compared to ensure accuracy and reproducibility.

Selection criteria and outcome evaluations

The title and abstract of the studies were screened by three colleagues, followed by an evaluation of the full text of the articles to identify relevant studies. Studies were considered eligible if they focused on the role of small molecules as epigenetic modulators in the autophagy mechanism associated with colorectal cancer and the potential of targeting autophagy as a therapeutic approach for colorectal cancer. Studies that focused on other types of cancers, studies without focusing on small molecules as epigenetic modulators or autophagy, review articles, and studies in other languages were excluded. There were no restrictions on the method of the study, age, gender, location, or study duration.

Article screening was conducted independently by two reviewers based on eligibility criteria. Any articles where reviewers disagreed on inclusion versus exclusion were marked as 'unsure' rather than unilateral decisions. A third senior reviewer then independently screened all 'unsure' articles blinded to initial decisions. The third reviewer's decision was considered final in case of continued disagreement between initial reviewers. Any uncertainties in interpreting eligibility were resolved through discussion between the three reviewers. This process ensured the systematic resolution of discrepancies between reviewers. To ensure the accuracy of data extraction, an Excel sheet was used to summarize the methods and results of the studies. The extracted data included: Study ID - A unique identifier code for each study, Author and publication year, Study design - Classified as in vitro, in vivo or clinical, Cancer cell line/tissue - The specific cells or tumors investigated, Sample size - Number of experimental groups or participants, Small molecule investigated - Name and a class of epigenetic modulator, Epigenetic modification targeted - E.g.

acetylation, methylation, Autophagy marker(s) assessed - E.g. LC3, Beclin1, p62, Technique(s) for outcome measurement - E.g. western blot, IHC, Main findings - A summary of results and conclusions draw. The methodological quality of each study was rigorously evaluated using Version 2 of the Cochrane risk-of-bias tool (RoB 2) to ensure the integrity of the review. The RoB 2 tool assesses the risk of bias in randomized trials across five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in the selection of the reported result. For non-randomized studies, the RoB 2 tool assesses bias due to confounding variables, bias in the selection of participants into the study, bias in the measurement of interventions, bias due to departures from intended interventions, bias due to missing data, bias in the measurement of outcomes, and bias in the selection of the reported result. Each domain was assigned a risk of bias judgment of low, some concerns, or high. Studies with a low risk of bias for all domains were judged as having a low risk of bias overall. Studies with a high or some concerns risk of bias in one or more domains but not in others were judged as having some concerns regarding the risk of bias overall. Studies with a high risk of bias in one or more domains were judged as having a high risk of bias overall (Sterne JAC 2019). Four studies were determined to have a high risk of bias due to inadequately reporting the randomization process and deviations from intended interventions (selection bias), as well as missing outcome data (attrition bias) in one study. Two additional studies had some concerns regarding the risk of bias relating to potential confounding, as they were non-randomized studies that did not fully adjust for prognostic factors.

A meta-analysis was not conducted due to several limitations of the available evidence. Only eight studies met the eligibility criteria, which is below the minimum number generally recommended for a robust meta-analysis. The included studies also had significant heterogeneity in terms of study designs, cell lines, small molecules investigated, and methods used to assess autophagy and outcomes. Given this diversity, it would not have been appropriate to pool results quantitatively. Furthermore, the studies measured different endpoints related to autophagy activation/inhibition and anticancer effects, precluding a meaningful combined analysis. The objectives of this review were to provide a qualitative narrative synthesis and discussion to explore mechanisms, rather than a quantitative meta-analysis.

Results

Study design and study characteristics

According to the search approach, 508 studies were found. After the removal of duplicates and non-relevant records based on the article title, 342 articles remained for further screening. Full-text screening was performed for studies, of which 8 studies were finally included. Figure 1 describes the PRISMA 2020 flow diagram of the qualifying research and search method.

Among eight remaining articles, 4 studies revealed the effects of small molecules on autophagy through epigenetic acetylation/deacetylation in CRC (Table 1). The four other studies focused on some other small molecules, which affected autophagy by epigenetic DNA methylation/demethylation and miRNA in CRC (Table 2).

As shown in Tables 1 and 2, the included articles consisted of 4 in vitro (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Ragusa et al. 2023) and 4 in vitro/in vivo studies (Gandesiri et al. 2012; Wu et al. 2015; Qiao et al. 2020; Yang et al. 2013). It is noteworthy that only one of these publications also contained human investigations (Yang et al. 2013). The obtained information showed that all mentioned studies used various CRC cell lines to evaluate their hypotheses.

Most of the included studies (6 articles) used the HCT116 CRC cell line to evaluate how small molecules affect autophagy through epigenetic alterations (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Gandesiri et al. 2012; Yang et al. 2013; Qiao et al. 2020). Other CRC cell lines including, HT29 (three articles) (Chen et al. 2019; Wu et al. 2015; Qiao et al. 2020), SW480 (one article) (Sun et al. 2017) and Caco-2 (one article) (Ragusa et al. 2023) were also considered in the investigated studies.

On the other hand, xenograft animal models (in-vivo research) were employed in 4 investigations using male NMRI mice aged 6–8 weeks (Gandesiri et al. 2012), and old female BALB/cAnN.Cg-Foxn1nu/CrlNarl (Wu et al. 2015), male Balb/c nude mice (Yang et al. 2013), and animal model of azoxymethane (AOM)-induced CRC (Qiao et al. 2020). One of the extracted articles used tumor specimens obtained from four patients with CRC (Yang et al. 2013). The small molecules in 6 articles activated the autophagy pathway through epigenetic modulations (Chen et al. 2019; Jo et al. 2018; Gandesiri et al. 2012; Wu et al. 2015; Ragusa et al. 2023; Yang et al. 2013), whereas two other articles reported the inhibitory effects of small molecules as epigenetic modulators of autophagy (Sun et al. 2017; Qiao et al. 2020). These studies have assessed Beclin-1 (Sun et al. 2017; Gandesiri et al. 2012; Ragusa et al. 2023; Qiao et al. 2020), LC3II/LC3I (Sun et al. 2017; Chen et al. 2019; Gandesiri et al. 2012;

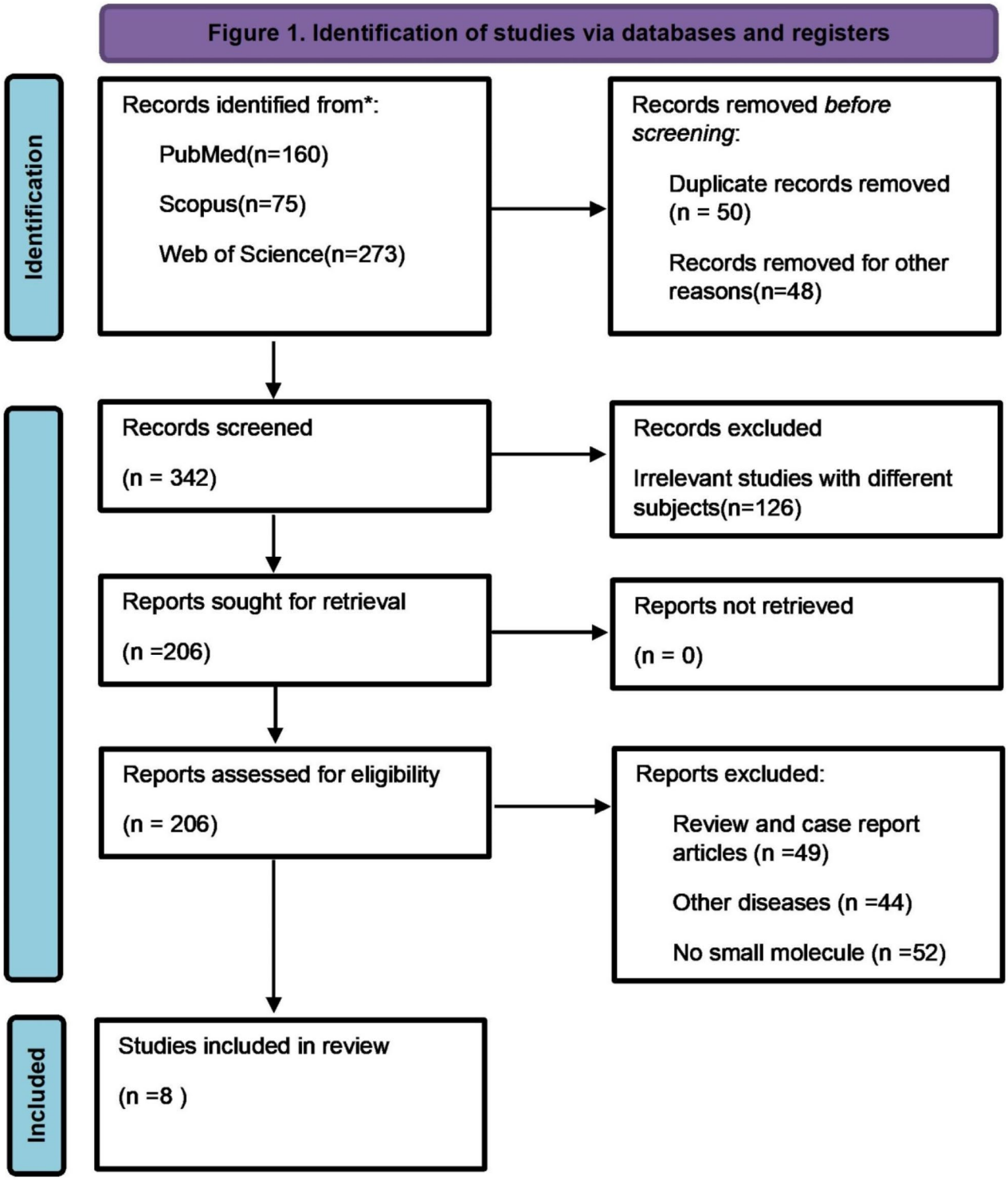

Fig. 1 The PRISMA 2020 flow diagram of the qualifying research and search method

Table 1 Small molecule-mediated activation/inhibition of autophagy through epigenetic modulation of histone acetylation/deacetylationin CRC

Author	Year	Type of study	Cell line/ animal model	Analysis technique	Small molecule	Epigenetic modulation	Autophagy marker	Autophagy activation/ inhibition	QA
Sun T	2017	In-vitro	HCT116 SW480	Western blot Fluorescence microscopy TEM	Aspirin	Beclin 1 acetylation	Beclin-1 LC3II/LC3I P62	Autophagy induction/ Autophagy flux inhibition	High risk
Chen MC	2019	In-vitro	HCT116 HT-29	Western Blot	MPT0G612	HDAC6 inhibition	ATG5 LC3II/LC3I P62	Activation	High risk
Jo YK	2018	In-vitro	HCT116	Western Blot	Valproic acid Suberoyl-anilide hydroxamic acid (SAHA) CI994	HDAC inhibition	UVRAG ATG4 ATG6 ATG7 ATG10	Activation	Low risk
Gandesiri M	2012	In-vitro/ In-vivo	HCT116 Xenograft model (6–8 week old male NMRI mice)	Western blot Immuno-fluorescence staining Acridine orange staining	Panobinostat (LBH589)	HDAC inhibition Induction of H3/H4 acetylation	LC3II/LC3I P62 Beclin1 ATG7	Activation	Low risk

TEM: Transmission electron microscopy, LC3: light chain 3, HDACs: Histone deacetylases, ATGs: Autophagy-related genes, UVRAG: UV Radiation Resistance Associated, NMRI: Naval Medical Research Institute

Table 2 Small molecule-mediated activation/inhibition of autophagy through epigenetic modulation of DNA methylation/demethylation and miRNA in CRC

Author	Year	Type of study	Cell line/animal model	Analysis technique	Small molecule	Epigenetic modulation	Autophagy marker	Autophagy activation/inhibition	QA
Wu JC	2015	In-vitro/ In-vivo	HT-29 Xenograft model (5 week old female BALB/cAnN. Cg-*Foxn1nu*/CrlNarl)	Western blot	Se-allylselenocysteine (ASC)	PCDH17 up-regulation by DNMT1 inhibition	LC3II/ LC3I	Activation	High risk
Ragusa MA	2023	In-vitro	Caco-2	Western Blot Flow cytometry	Indicaxanthin	DNMT inhibition	LC3II/ LC3I Beclin1 Autophagosome formation	Activation	High risk
Yang PM	2013	In-vitro/ In-vivo Human study	HCT116 Xenograft model (male Balb/c nude mice) CRC patients	Western Blot Microarray	Zebularine (Zeb)	P53 up-regulation by DNMT inhibition	LC3II/ LC3I P62	Activation	Some concerns
Qiao PF	2020	In-vitro/ In-vivo	HCT116 HT-29 Rat model of azoxymethane (AOM)-induced CRC	Western blot	Catalpol	SIRT1 inhibition by mir-34a up-regulation	Beclin-1 LC3II/ LC3I	Inhibition	Some concerns

PCDH17: protocadherin 17 gene, DNMT: DNA methyltransferase, CRC: Colorectal cancer, SIRT: Sirtuins

Wu et al. 2015; Ragusa et al. 2023; Yang et al. 2013; Qiao et al. 2020), P62 (Sun et al. 2017; Chen et al. 2019; Gandesiri et al. 2012; Yang et al. 2013), ATG5 (Chen et al. 2019), ATG4, ATG6, ATG7, ATG10 and UVRAG (Jo et al. 2018) as the most important autophagy markers. The techniques used to examine these markers include western blot (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Gandesiri et al. 2012; Wu et al. 2015; Ragusa et al. 2023; Yang et al. 2013; Qiao et al. 2020), immune-florescent staining (Gandesiri et al. 2012), Acridine orange staining (Gandesiri et al. 2012), Transmission electron microscopy (TEM) (Sun et al. 2017), microarray (Yang et al. 2013), and flow cytometry (Ragusa et al. 2023).

These studies revealed that small molecules used for cancer therapy may affect the autophagy signaling pathway and cancer cell fate by epigenetic modulations, such as acetylation/deacetylation (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Gandesiri et al. 2012), DNA methylation/demethylation (Wu et al. 2015; Ragusa et al. 2023; Yang et al. 2013), and miRNA (Qiao et al. 2020).

Small molecules affect autophagy through epigenetic modulation of histone acetylation/deacetylation in CRC

We have identified 5 small molecules studied in 4 articles and one small molecule in another study, which respectively activated and inhibited the autophagy in CRC cell lines through modulation of histone acetylation/deacetylation (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Gandesiri et al. 2012). Autophagy activation or inhibition by these small molecules differently affected their anticancer activity.

For instance, panobinostat (LBH589) as an HDAC inhibitor up-regulated the tumor suppressor death-associated protein kinase (DAPK) in the HCT116 cell line. Panobinostat consequently induced DAPK-dependent autophagy and led cells to apoptosis (Gandesiri et al. 2012).

In contrast, autophagy induction by some small molecules led to the survival of cancer cells.

For instance, autophagy activation by an HDAC6 inhibitor, MPT0G612, resulted in pro-survival signals in the HCT116 cell line and decreased MPT0G612-Induced apoptosis. Therefore, co-treatment with an autophagy inhibitor such as chloroquine (CQ) may enhance MPT0G612-mediated apoptotic cell death (Chen et al. 2019). On the other hand, three HDAC1 inhibitors including Valproic acid, Suberoylanilide hydroxamic acid (SAHA) and CI994 attenuated 5FU-mediated cell death following the induction of UVRAG-mediated autophagy (Jo et al. 2018). It seems that co-treatment with autophagy inhibitors may also increase the anticancer effect of these small molecules. Among all evaluated small molecules, that affected autophagy through epigenetic acetylation/deacetylation, aspirin was the only one that inhibited autophagy (Sun et al. 2017). Aspirin induced Beclin1 acetylation, which although induced autophagy induction, also inhibited autolysosome destruction. Consequently, the anticancer activity of aspirin was hampered by inhibition of autophagy flux. Interestingly, co-treatment of aspirin with another small molecule C646 enhanced its cytotoxic effect on CRC cells by inhibiting the acetyltransferase p300-mediated acetylation of Beclin1(Sun et al. 2017). Figure 2 has summarizes how investigated small molecules affected autophagy by epigenetic modulation of histone acetylation/deacetylation in CRC.

Small molecule epigenetic modulators affect autophagy in CRC through DNA methylation/demethylation and miRNA

We have identified 4 articles in which 4 small molecules affected the autophagy in CRC cell lines by epigenetic modulation of DNA methylation/demethylation and miRNA (Wu et al. 2015; Ragusa et al. 2023; Yang et al. 2013).

In this regard, Se-allylselenocysteine (ASC) (Wu et al. 2015), Zebularine (Zeb) (Yang et al. 2013), and Indicaxanthin (Ragusa et al. 2023) inhibited the DNA methyltransferases (DNMTs) and decreased the promoter methylation of PCDH17, P53 and Beclin1/ LC3II, respectively.

Wu JC et al. demonstrated that ASC induced autophagic HT-29 cell death by methylation regulation of PCDH17 expression and AMPK/mTOR pathway (Wu et al. 2015). Yang PM et al. revealed that the Zeb suppressed colorectal tumorigenesis and stemness by induction of p53-dependent endoplasmic reticulum stress and autophagy cell death in the HCT116 cell line (Yang et al. 2013). Indicaxanthin also demethylated autophagy-related genes (*Beclin1*, *LC3II*) and resulted in improved anticancer effect of this small molecule in the Caco-2 cell line (Ragusa et al. 2023). Unlike other small molecules, in the study conducted by Qiao PF et al., catalpol inhibited autophagy by increasing the miR-34a expression level and consequently down-regulation of SIRT1. The catalpol-mediated miR-34a/SIRT1 axis suppressed autophagy and malignancy behavior in HCT116 and HT-29 cell lines (Qiao et al. 2020).

Therefore, autophagy activation/inhibition mediated by different small molecules has contrary effects on their anticancer potential, which should be considered in their application for CRC therapy. Figure 3 describes the effect of investigated small molecules on autophagy by epigenetic modulation of DNA methylation/demethylation and miRNA in CRC.

Discussion

In this systematic review, we deeply analyzed the available literature on the effect of small molecules as epigenetic modulators of the autophagy pathway in CRC. This type of cancer with high incidence and mortality along with drug resistance is considered a great public health concern (Wang et al. 2022a).

Evidence indicated that epigenetic modifications such as DNA methylation/demethylation, histone acetylation/deacetylation and gene expression regulation by miRNAs are considered hallmarks of cancers such as CRC by regulation of important genes involved in the autophagy pathway (Ghavami et al. 2022). For instance, the promoter

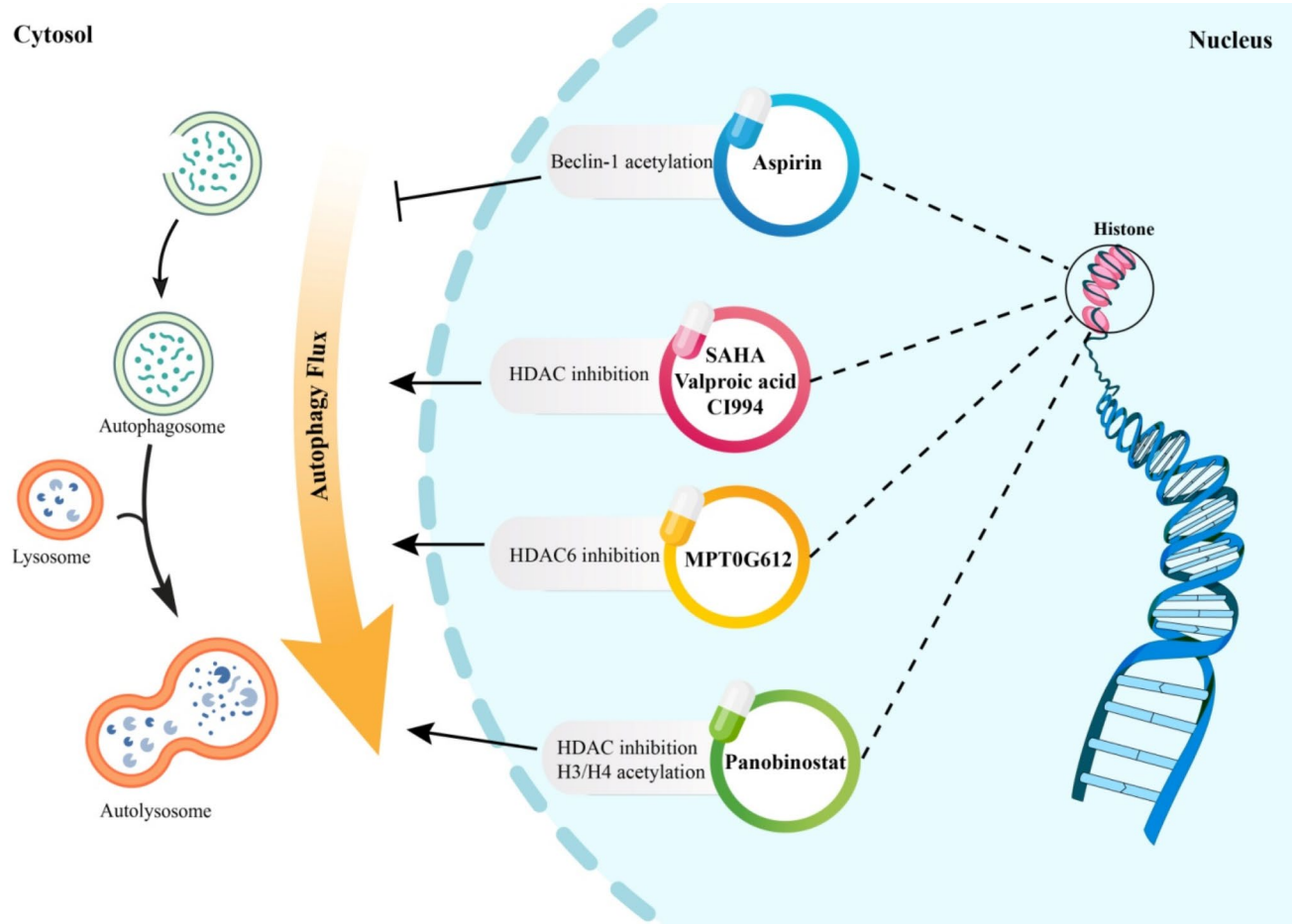

Fig. 2 The effect of small molecules on autophagy by epigenetic modulation of histone acetylation/deacetylation in CRC. The autophagy activation following the HDAC inhibition by small molecules may promote cancer cell death (Panobinostat) or survival (MPT0G612, SAHA, Valproic acid, and CI994). The autophagic flux inhibition following the *Beclin1* acetylation by Aspirin decreased the anticancer activity of this small molecule

hypermethylation of the Tumor-suppressing effect of the B-cell translocation gene (BTG) results in autophagy inhibition and promotes CRC malignant transformation (Bhol et al. 2020; Zhao et al. 2017). Histone deacetylase 1 (HDAC1) binds to autophagy-related 16 like 1 (ATG16L1) and reduces its acetylation. Deacetylation of the ATG16L1 as a core protein of autophagy enhances its interaction with ATG12-ATG5 conjugate and consequently activates pro-survival autophagy in CRC (HDAC1 promotes basal autophagy and proliferation of colorectal cancer cells by mediating ATG16L1 deacetylation). The regulation of autophagy by miRNAs plays an important role in CRC tumor suppression or promotion and also treatment resistance (Fesler et al. 2017). The elevated level of miR-183-5p in CRC inhibits autophagy by targeting ATG5 and promote poor prognosis (Zheng et al. 2019). On the other hand, miR-22 switches autophagy to apoptosis by targeting BTG1 and sensitizes CRC cells to 5-fluorouracil (5-FU) (Zhang et al. 2015).

To find novel strategies for CRC treatment, targeting epigenetic modifications by different small molecules has been considered till now (Wang et al. 2021; Xiao et al. 2021; Kumar et al. 2023).

Different molecular mechanisms by which small molecules modulate epigenetic changes and autophagy are considered including, complex interactions between chromatin remodeling, transcriptional regulation, and post-translational modifications of autophagy-related proteins (Jin et al. 2022). For instance, histone-modifier enzymes, such as histone methyltransferases (HMTs) and histone deacetylases (HDACs) are targeted by small molecules, which modify the chromatin structure and accessibility of DNA to transcription factors and consequently gene expression level (Wang et al. 2021). Small molecules can affect the methylation status of the promoter CpG islands and the gene expression level of the autophagy-related genes by targeting DNA methyltransferase enzymes (Wang et al. 2021). Small molecules can alter miRNA expression regulation,

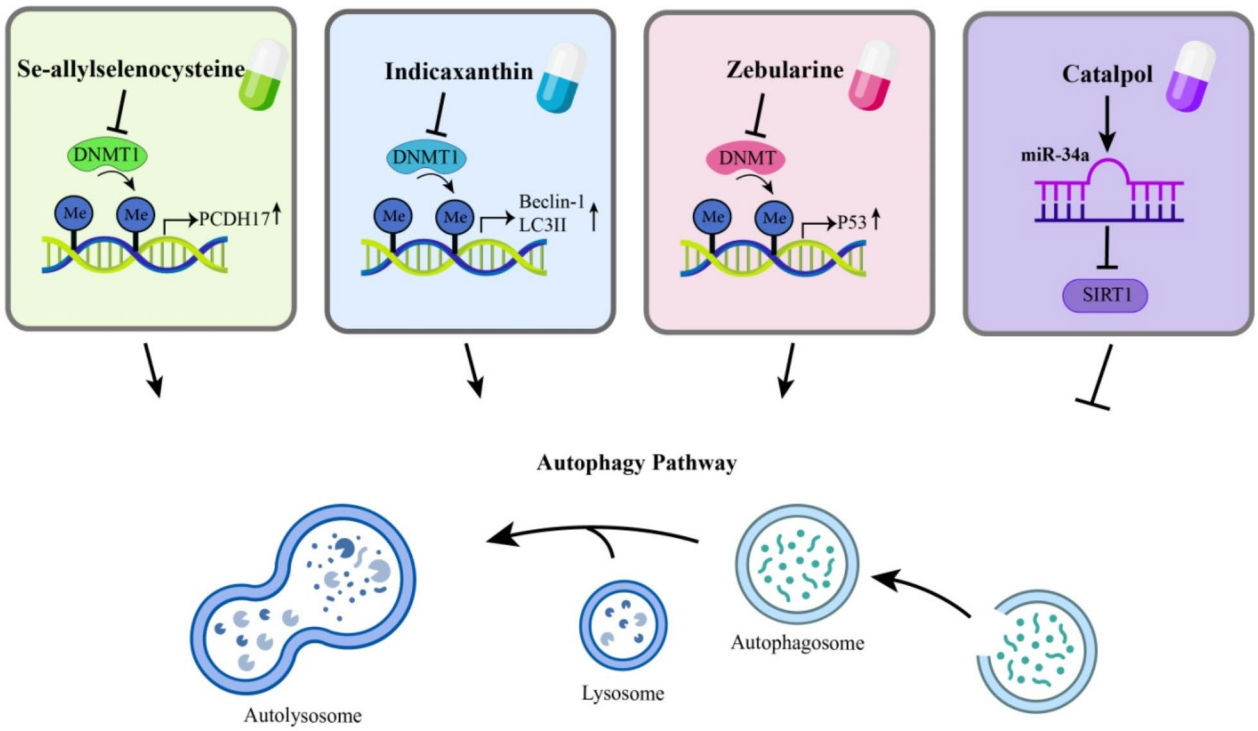

Fig. 3 The effect of small molecules on autophagy by epigenetic modulation of DNA methylation/demethylation and miRNA in CRC. The autophagy activation following the DNMT inhibition by small molecules including Se-allylselenocysteine, Indicaxanthin, and Zebularine resulted in CRC cell death. The autophagy inhibition by catalpol-mediated miR-34a/SIRT1 axis suppressed the malignancy behavior in CRC cells

miRNA biogenesis, and miRNA-mRNA interactions, which finally affect the post-transcriptional regulation of autophagy-related genes by specific miRNAs (Jing et al. 2015; Lei et al. 2022). Some small molecules can also epigenetically modulate signaling pathways, which interact and regulate autophagy pathway such as mTOR, AMPK, and PI3K/Akt pathways (Kondapuram et al. 2019).

In one study, compounds 3b and 4a inhibited the HCT116 CRC cell proliferation by targeting the DNA methyltransferase (DNMT) enzyme (Pechalrieu et al. 2020). In another study, MHY2256 acts as an HDAC-SIRT inhibitor and leads to cell cycle arrest, induction of apoptosis and autophagic cell death in the HCT116 CRC cell line (Kim et al. 2020). A natural small molecule, curcumin, also downregulates miR-130a and consequently suppresses CRC cell proliferation by inhibiting Wnt/β-Catenin Pathways (Dou et al. 2017).

On the other hand, autophagy is known as a double-edged sword in CRC and regulation of this signaling pathway may be a novel strategy in CRC treatment especially in drug-resistant types (Zhang and Liu 2021). Different pharmacological small molecules targeted at autophagy have been considered to treat CRC (Li et al. 2022). For instance, activation of mitogen-activated protein kinase) MAPK by a small lipophilic molecule, Cannabidiol (CBD), resulted in paraptosis, apoptosis, autophagy and eventually CRC cell death (Kim et al. 2024). Another small molecule, S130, as an inhibitor of autophagy-related gene 4B (ATG4B) promoted CRC cell death by autophagy suppression (Fu et al. 2019).

The importance of both epigenetic modifications and autophagy signaling pathway in CRC led to some studies, which consider the epigenetic regulation of autophagy in this type of cancer (Ghavami et al. 2022; Zandieh et al. 2023). As discussed in previous review studies, various small molecules are considered targeted therapeutic agents in CRC by targeting each epigenetic modification (Wang et al. 2021; Xiao et al. 2021; Kumar et al. 2023) or autophagy pathway (Limpert et al. 2018; Zhang et al. 2018, 2021; Kondapuram et al. 2019). However, to the best of our knowledge, no study has systematically reviewed small molecules as specific epigenetic modulators of autophagy in CRC or other cancers.

The importance of epigenetic regulation of autophagy in tumorigenesis and anti-tumorigenesis, and the potential role

of small molecules on both epigenetic modifications and autophagy, attracted us to perform this systematic review for a better understanding of the action mechanism of small molecule epigenetic modulators on autophagy in CRC. On the other hand, the dual role of autophagy in CRC tumor suppression or promotion complicates the therapeutic strategies. The contrasting roles of small molecules in activating or inhibiting the autophagy pathway also complicate the CRC therapeutics strategies. Therefore, it seems necessary to perform systematic studies to better comprehend and interpret the results.

For this purpose, after accurate screening and assessment of related articles, a total 8 eligible studies on CRC were included in this systematic review. These studies focused on the epigenetic role of 10 small molecules including, aspirin (Sun et al. 2017), MPT0G612 (Chen et al. 2019), valproic acid (Jo et al. 2018), Suberoylanilide hydroxamic acid (SAHA) (Jo et al. 2018), CI994 (Jo et al. 2018), Panobinostat (LBH589) (Gandesiri et al. 2012), Se allylselenocysteine (ASC) (Wu et al. 2015), Indicaxanthin (Ragusa et al. 2023), Zebularine (Zeb) (Yang et al. 2013), and Catalpol (Qiao et al. 2020) on autophagy pathway in CRC.

The obtained results revealed that some small molecules such as Panobinostat (LBH589) induced autophagy through HDAC inhibition, which remarkably improved CRC cell death (Gandesiri et al. 2012). In addition, other small molecules including Se allylselenocysteine (ASC) (Wu et al. 2015), Indicaxanthin (Ragusa et al. 2023), and Zebularine (Zeb) (Yang et al. 2013) also activated the autophagy by inhibition of DNMT enzymes. Inhibition of DNMT enzymes resulted in hypomethylation and increased expression of autophagy-related genes and finally improved cancer cell death.

In contrast, autophagy inhibition may have a dual role in the anticancer effect of small molecules. For instance, catalpol enhanced the miR-34a expression level and consequently decreased SIRT1 as a positive regulator of autophagy. Autophagy inhibition by catalpol improved its cytotoxicity to cancer cells (Qiao et al. 2020). On the other hand, autophagy flux inhibition by another small molecule, aspirin led to cancer cell prosurvival, which indicated the necessity of using a combination therapy of aspirin with another small molecule C646 as an autophagy inhibitor (Sun et al. 2017).

According to the evidence, it can be concluded that different small molecules epigenetically affect the activity of the autophagy pathway. Due to the contrasting role of autophagy in tumor progression and suppression, the administration of small molecules with different effects on autophagy may variously influence the fate of cancer cells and their response or resistance to drugs. These effects complicate the therapeutic strategies and the application of small molecules to target autophagy in CRC may have both beneficial and adverse effects due to the dual role of autophagy (Wu et al. 2024). Therefore it seems that there are some potential risks of targeting the autophagy pathway with small molecules. For instance, the pro-survival and pro-death autophagic pathways may be switched to each other in tumor cells in response to various treatments. This dual role may contribute to therapeutic resistance and therefore, it will be challenging to effectively predict and target autophagy in the clinic (Qin et al. 2023).

Cancer cells also develop resistance mechanisms to adapt to tumor microenvironment changes and evade autophagy-mediated cell death. The long-term and improper autophagy modulation with small molecules may contribute to surviving resistant cells within the tumor and lead to treatment failure and poorer clinical outcomes (Towers et al. 2019).

Considering the complex role of autophagy in cancer metastasis through affecting cell migration, invasion, and epithelial-mesenchymal transition (EMT), autophagy dysregulation by small molecules may promote cancer metastasis and progression (Manzoor et al. 2022). The critical role of autophagy in regulating immune responses, autophagy modulation with small molecules may disrupt immune cell function and make immunosuppressive tumor microenvironment, which promotes immune evasion and tumor growth (Mukhopadhyay et al. 2022). Autophagy plays a key role in maintaining genomic stability by removing damaged proteins and organelles, which prevents the accumulation of mutations-mediated tumorigenesis. Targeting autophagy by small molecules may enhance genetic instability and promote tumor growth and progression (Kondapuram et al. 2019). Due to the role of autophagy in the maintenance and self-renewal of cancer stem cells, autophagy modulation with small molecules may also enhance the survival of cancer stem cells and therapeutic resistance (Wang et al. 2022b).

The outcome effect of autophagy modulation in the progression or treatment of cancer is highly context-dependent, and is influenced by factors such as tumor stage, tumor microenvironmental conditions, genetic modification, and also type of treatment regimens. Given this complexity, it is crucial to consider the specific context of autophagy dysregulation in individual patients and therapeutic strategies should be designed accordingly (Bhat et al. 2018; Assi and Kimmelman 2023).

To manage the above-mentioned complexities some points should be considered. For instance, the context-specific effects of small molecules on the autophagy pathway should be evaluated. Understanding the cellular environment, specifically involved signaling pathways and mechanisms by which small molecules modulate autophagy is essential. The clarification of the context-dependent effects

contributes to improving autophagy-based therapeutic strategies based on the specific disease and patient condition (Zhang et al. 2018). Another approach to adopt the dual role of small molecules in autophagy modulation is combination therapy using an autophagy inducer along with an autophagy inhibitor. This strategy can make a balanced autophagy regulation, which improves the therapeutic effect and reduces the adverse effects of each drug alone (Liu et al. 2020). The identification of the exact molecular targets and downstream effects of small molecule autophagy modulators contributes to detecting specific proteins and pathways that mediate the therapeutic response. Having this knowledge can be a suitable guide to designing more selective autophagy modulators with maximal therapeutic effects and minimal unwanted side effects (Whitmarsh-Everiss and Laraia 2021).

Precision medicine principles will be helpful to develop individualized treatment strategies based on the patient's unique characteristics such as genetic background, features of the disease, and autophagy status. Identification of the proper biomarkers and molecular profiling techniques recognizes patient candidates for achieving autophagy modulators as therapeutic strategies (Lim, Mohamad Hanif and Chin 2021).

On the other hand, regular monitoring of the autophagy activation during the treatment period using imaging techniques or biomarker assessments enables real-time following of the effect of the autophagy modulation on disease progression and treatment response or resistance (Ding and Hong 2020).

While this systematic review provides useful insights into the effects of small molecules as epigenetic modulators of autophagy in colorectal cancer, some limitations must be acknowledged. The primary limitation is the small number of studies that were eligible for inclusion. Only eight studies met our selection criteria, limiting the conclusions that can be drawn. Another limitation is the lack of human studies exploring this topic. All included studies relying solely on in vitro or animal models. While preclinical models are important for initial investigation, it is difficult to generalize findings to human cancers without human subject research. Differences between animal and human biology could mean the effects we observed may not generalize to humans. The types of study designs included were also limited with most being in vitro experiments conducted using CRC cell lines. Cell line work provides a controlled environment but cannot fully recapitulate the complexity of an in vivo tumor. The inclusion of only a few animal studies and lack of clinical trials means we could not assess the effects in living systems or the potential for clinical application. Additionally, the studies used different small molecules, cancer cell lines, and methods for evaluating autophagy and response (Sun et al. 2017; Chen et al. 2019; Jo et al. 2018; Ragusa et al. 2023; Gandesiri et al. 2012; Wu et al. 2015; Qiao et al. 2020; Yang et al. 2013). This heterogeneity limits our ability to pool and directly compare the results.

Taken together, this systematic review confirmed that the dual role of the autophagy pathway in CRC tumor promotion and suppression complicates the application of small molecules in CRC treatment. This study can give a clue about the direction and trend of future studies that will help to better understand how small molecules should be administered to have the best effect and the least side effects in CRC therapy. Therefore, more comprehensive preclinical studies are suggested for future research focused on combination therapies, personalized treatment, biomarker analysis and exact monitoring of the treatment responses to manage small molecule-based cancer therapy strategies.

Conclusion

Taken together, these results suggest that the development of epigenetic modulators of autophagy holds great promise for current CRC therapy due to numerous regulatory targets found within this pathway and their crucial role in drug resistance and carcinogenesis. However, considering the dual role of autophagy, it is necessary to carefully notice the potential risks and undesirable consequences of autophagy modulation by small molecules in cancer therapy. Conducting further studies with larger sample sizes and clinical trials on the epigenetic effect of various small molecules on CRC as well as other cancers and also human evaluations will be necessary for a more accurate conclusion.

Author contributions P.M., M.Z. and S.D. made a substantial contribution to the conception and design of the work, interpretation of the data and drafting of the work. F.S., M.S., S.I., N. Kh., M.Z., and S.D. were involved in the acquisition and analysis of the data and critical revision of the work for important intellectual content. M.S. provided the figures. All authors have given final approval of the version to be published and agree to be accountable for all aspects of the work.

Funding No funding was received for conducting this study.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Assi M, Kimmelman AC (2023) Impact of context-dependent autophagy states on tumor progression. Nat cancer 4:596–607

Bajpai P, Agarwal S, Afaq F, Al Diffalha S, Chandrashekar DS, Kim H-G, Shelton A, Miller CR, Singh SK, Singh R (2024) Combination of dual JAK/HDAC inhibitor with regorafenib synergistically reduces tumor growth, metastasis, and regorafenib-induced toxicity in colorectal cancer. J Experimental Clin Cancer Res 43:192

Bhat P, Kriel J, Priya BS, Shivananju NS, Loos B (2018) Modulating autophagy in cancer therapy: advancements and challenges for cancer cell death sensitization. Biochem Pharmacol 147:170–182

Bhol CS, Panigrahi DP, Praharaj PP, Mahapatra KK, Patra S, Mishra SR, Behera BP, Bhutia SK (2020) Epigenetic modifications of autophagy in cancer and cancer therapeutics. Seminars in cancer biology. Elsevier, pp 22–33

Chen M-C, Lin Y-C, Liao Y-H, Liou J-P, Chen C-H (2019) MPT0G612, a novel HDAC6 inhibitor, induces apoptosis and suppresses IFN-γ-induced programmed death-ligand 1 in human colorectal carcinoma cells. Cancers 11:1617

Darwiche N (2020) Epigenetic mechanisms and the hallmarks of cancer: an intimate affair. Am J cancer Res 10:1954

Debnath J, Gammoh N, Ryan KM (2023) Autophagy and autophagy-related pathways in cancer. Nat Rev Mol Cell Biol 24:560–575

Ding S, Hong Y (2020) The fluorescence toolbox for visualizing autophagy. Chem Soc Rev 49:8354–8389

Dou H, Shen R, Tao J, Huang L, Shi H, Chen H, Wang Y, Wang T (2017) Curcumin suppresses the colon cancer proliferation by inhibiting Wnt/β-catenin pathways via miR-130a. Front Pharmacol 8:877

Du Y-X, Mamun AA, Lyu A-P, Zhang H-J (2023) Natural compounds targeting the autophagy pathway in the treatment of colorectal cancer. Int J Mol Sci 24:7310

Duncan EJ, Gluckman PD, Dearden PK (2014) Epigenetics, plasticity, and evolution: how do we link epigenetic change to phenotype? J Experimental Zool Part B: Mol Dev Evol 322:208–220

Far BF, Vakili K, Fathi M, Yaghoobpoor S, Bhia M, Naimi-Jamal MR (2023) The role of microRNA-21 (miR-21) in pathogenesis, diagnosis, and prognosis of gastrointestinal cancers: a review. Life Sci 316:121340

Fesler A, Liu H, Wu N, Liu F, Ling P, Ju J (2017) Autophagy regulated by miRNAs in colorectal cancer progression and resistance. Cancer Translational Med 3:96

Fu Y, Hong L, Xu J, Zhong G, Gu Q, Gu Q, Guan Y, Zheng X, Dai Q, Luo X (2019) Discovery of a small molecule targeting autophagy via ATG4B inhibition and cell death of colorectal cancer cells in vitro and in vivo. Autophagy 15:295–311

Gandesiri M, Chakilam S, Ivanovska J, Benderska N, Ocker M, Di Fazio P, Feoktistova M, Gali-Muhtasib H, Rave-Fränk M, Prante O (2012) DAPK plays an important role in panobinostat-induced autophagy and commits cells to apoptosis under autophagy deficient conditions. Apoptosis 17:1300–1315

Ghavami S, Zamani M, Ahmadi M, Erfani M, Dastghaib S, Darbandi M, Darbandi S, Vakili O, Siri M, Grabarek BO (2022) Epigenetic regulation of autophagy in gastrointestinal cancers. Biochim et Biophys Acta (BBA)-Molecular Basis Disease 1868:166512

Hammond WA, Swaika A, Mody K (2016) Pharmacologic resistance in colorectal cancer: a review. Therapeutic Adv Med Oncol 8:57–84

Housini M, Dariya B, Ahmed N, Stevens A, Fiadjoe H, Nagaraju GP, Basha R (2023) Colorectal cancer: genetic alterations, novel biomarkers, current therapeutic strategies and clinical trails. Gene, 147857

Hu L-F (2019) Epigenetic regulation of autophagy. Autophagy: Biology Diseases: Basic Sci, 221–236

Ichimiya T, Yamakawa T, Hirano T, Yokoyama Y, Hayashi Y, Hirayama D, Wagatsuma K, Itoi T, Nakase H (2020) Autophagy and autophagy-related diseases: a review. Int J Mol Sci 21:8974

Jin Y, Liu T, Luo H, Liu Y, Liu D (2022) Targeting epigenetic regulatory enzymes for cancer therapeutics: novel small-molecule epidrug development. Front Oncol 12:848221

Jing Z, Han W, Sui X, Xie J, Pan H (2015) Interaction of autophagy with microRNAs and their potential therapeutic implications in human cancers. Cancer Lett 356:332–338

Jo YK, Park NY, Shin JH, Jo DS, Bae J-E, Choi ES, Maeng S, Jeon HB, Roh SA, Chang JW (2018) Up-regulation of UVRAG by HDAC1 inhibition attenuates 5FU-induced cell death in HCT116 colorectal cancer cells. Anticancer Res 38:271–277

Karmacharya U, Jung J-W (2023) Small molecule inhibitors for unc-51-like autophagy-activating kinase targeting autophagy in cancer. Int J Mol Sci 24:953

Kim MJ, Kang YJ, Sung B, Jang JY, Ahn YR, Oh HJ, Choi H, Choi I, Im E, Moon HR (2020) Novel SIRT inhibitor, MHY2256, induces cell cycle arrest, apoptosis, and autophagic cell death in HCT116 human colorectal cancer cells. Biomolecules Ther 28:561

Kim NY, Mohan CD, Sethi G, Ahn KS (2024) Cannabidiol activates MAPK pathway to induce apoptosis, paraptosis, and autophagy in colorectal cancer cells. J Cell Biochem

Kondapuram SK, Sarvagalla S, Coumar MS (2019) Targeting autophagy with small molecules for cancer therapy. J Cancer Metastasis Treat 5:1020517

Kumar A, Emdad L, Fisher PB, Das SK (2023) Targeting epigenetic regulation for cancer therapy using small molecule inhibitors. In *Advances in Cancer Research*, 73–161. Elsevier

Lei Y, Chen L, Liu J, Zhong Y, Deng L (2022) The MicroRNA-based strategies to combat cancer chemoresistance via regulating autophagy. Front Oncol 12:841625

Li Q, Kang C (2020) Mechanisms of action for small molecules revealed by structural biology in drug discovery. Int J Mol Sci 21:5262

Li Z, Si W, Jin W, Yuan Z, Chen Y, Fu L (2022) Targeting autophagy in colorectal cancer: an update on pharmacological small-molecule compounds. Drug Discovery Today 27:2373–2385

Liang H, He X, Tong Y, Bai N, Pu Y, Han K, Wang Y (2023) Ferroptosis open a new door for colorectal cancer treatment. Front Oncol 13:1059520

Lim SM, Mohamad EA, Hanif, Chin S-F (2021) Is targeting autophagy mechanism in cancer a good approach? The possible double-edge sword effect. Cell Bioscience 11:56

Limpert AS, Lambert LJ, Bakas NA, Bata N, Brun SN, Shaw RJ, Cosford ND (2018) Autophagy in cancer: regulation by small molecules. Trends Pharmacol Sci 39:1021–1032

Liu T, Zhang J, Li K, Deng L, Wang H (2020) Combination of an autophagy inducer and an autophagy inhibitor: a smarter strategy emerging in cancer therapy. Front Pharmacol 11:408

Manzoor S, Muhammad JS, Maghazachi AA, Hamid Q (2022) Autophagy: a versatile player in the progression of colorectal cancer and drug resistance. Front Oncol 12:924290

Moshawih S, Lim AF, Ardianto C, Goh KW, Kifli N, Goh HP, Jarrar Q, Ming LC (2022) Target-based small molecule drug discovery for colorectal cancer: a review of molecular pathways and in silico studies. Biomolecules 12:878

Mukhopadhyay S, Mahapatra KK, Praharaj PP, Patil S, Bhutia SK (2022) Recent progress of autophagy signaling in tumor microenvironment and its targeting for possible cancer therapeutics. Seminars in cancer biology. Elsevier, pp 196–208

Nagaraju GP, EI-Rayes BF (2013) SPARC and DNA methylation: possible diagnostic and therapeutic implications in gastrointestinal cancers. Cancer Lett 328:10–17

Nagaraju GP, Wu C, Merchant N, Chen Z, Lesinski GB, El-Rayes BF (2017) Epigenetic effects of inhibition of heat shock protein 90 (HSP90) in human pancreatic and colon cancer. Cancer Lett 402:110–116

Nagaraju GP, Kasa P, Dariya B, Surepalli N, Peela S, Ahmad S (2021) Epigenetics and therapeutic targets in gastrointestinal malignancies. Drug Discovery Today 26:2303–2314

Nalli M, Puxeddu M, La Regina G, Gianni S, Silvestri R (2021) Emerging therapeutic agents for colorectal cancer. Molecules 26:7463

Pan Z, Xie Y, Bai J, Lin Q, Cui X, Zhang N (2018) Bufalin suppresses colorectal cancer cell growth through promoting autophagy in vivo and in vitro. RSC Adv 8:38910–38918

Patnaik S (2019) Drugs targeting epigenetic modifications and plausible therapeutic strategies against colorectal cancer. Front Pharmacol 10:448742

Pechalrieu D, Dauzonne D, Arimondo PB, Lopez M (2020) Synthesis of novel 3-halo-3-nitroflavanones and their activities as DNA methyltransferase inhibitors in cancer cells. Eur J Med Chem 186:111829

Qiao PF, Yao L, Zeng ZL (2020) Catalpol–mediated microRNA–34a suppresses autophagy and malignancy by regulating SIRT1 in colorectal cancer. Oncol Rep 43:1053–1066

Qin Y, Ashrafizadeh M, Mongiardini V, Grimaldi B, Crea F, Rietdorf K, Győrffy B, Klionsky DJ, Ren J, Zhang W (2023) Autophagy and cancer drug resistance in dialogue: pre-clinical and clinical evidence. Cancer Lett, 216307

Ragusa MA, Naselli F, Cruciata I, Volpes S, Schimmenti C, Serio G, Mauro M, Librizzi M, Luparello C, Chiarelli R (2023) Indicaxanthin induces autophagy in intestinal epithelial cancer cells by epigenetic mechanisms involving DNA methylation. Nutrients 15:3495

Rakesh R, PriyaDharshini LC, Sakthivel KM, Rasmi RR (2022) Role and regulation of autophagy in cancer. Biochim et Biophys Acta (BBA)-Molecular Basis Disease 1868:166400

Rawla P, Sunkara T, Barsouk A (2019) Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterol Review/Przegląd Gastroenterologiczny 14:89–103

Sadida HQ, Abdulla A, Al Marzooqi S, Hashem S, Macha MA, Akil ASA-S, Bhat AA (2024) Epigenetic modifications: Key players in cancer heterogeneity and drug resistance. Translational Oncol 39:101821

Sterne JAC, Page SJ, Elbers MJ, Blencowe RG, Boutron NS, Cates I, Cheng CJ, Corbett H-Y, Eldridge MS, Hernán SM, Hopewell MA, Hróbjartsson S, Junqueira A, Jüni DR, Kirkham P, Lasserson JJ, Li T, McAleenan T, Reeves A, Shepperd BC, Shrier S, Stewart I, Tilling LA, White K, Whiting IR, Higgins PF (2019) JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ ; 366: l4898. (2019)

Sun T, Ming L, Yan Y, Zhang Y, Xue H (2017) Beclin 1 acetylation impairs the anticancer effect of aspirin in colorectal cancer cells. Oncotarget 8:74781

Szigeti KA, Kalmár A, Galamb O, Valcz G, Barták BK, Nagy ZB, Zsigrai S, Felletár I, Patai ÁV, Micsik T (2022) Global DNA hypomethylation of colorectal tumours detected in tissue and liquid biopsies may be related to decreased methyl-donor content. BMC Cancer 22:605

Towers CG, Wodetzki D, Thorburn A (2019) Autophagy and cancer: modulation of cell death pathways and cancer cell adaptations. J Cell Biol 219:e201909033

Wang Y, Xie Q, Tan H, Liao M, Zhu S, Zheng L-L, Huang H, Liu B (2021) Targeting cancer epigenetic pathways with small-molecule compounds: therapeutic efficacy and combination therapies. Pharmacol Res 173:105702

Wang Q, Shen X, Chen G, Du J (2022a) Drug resistance in colorectal cancer: from mechanism to clinic. Cancers 14:2928

Wang X, Lee J, Xie C (2022b) Autophagy regulation on cancer stem cell maintenance, metastasis, and therapy resistance. Cancers 14:381

Whitmarsh-Everiss T, Laraia L (2021) Small molecule probes for targeting autophagy. Nat Chem Biol 17:653–664

Wu JC, Wang FZ, Tsai ML, Lo CY, Badmaev V, Ho CT, Wang YJ, Pan MH (2015) Se-Allylselenocysteine induces autophagy by modulating the AMPK/mTOR signaling pathway and epigenetic regulation of PCDH17 in human colorectal adenocarcinoma cells. Mol Nutr Food Res 59:2511–2522

Wu Y, Wang A, Feng G, Pan X, Shuai W, Yang P, Zhang J, Ouyang L, Luo Y, Wang G (2024) Autophagy modulation in cancer therapy: challenges coexist with opportunities. Eur J Med Chem, 116688

Xiao W, Zhou Q, Wen X, Wang R, Liu R, Wang T, Shi J, Hu Y, Hou J (2021) Small-molecule inhibitors overcome epigenetic reprogramming for cancer therapy. Front Pharmacol 12:702360

Yan L, Shi J, Zhu J (2024) Cellular and molecular events in colorectal cancer: biological mechanisms, cell death pathways, drug resistance and signalling network interactions. Discover Oncol 15:1–29

Yang P-M, Lin Y-T, Shun C-T, Lin S-H, Wei T-T, Chuang S-H, Wu M-S, Chen C-C (2013) Zebularine inhibits tumorigenesis and stemness of colorectal cancer via p53-dependent endoplasmic reticulum stress. Sci Rep 3:3219

Yang Y, Liu P, Zhou M, Yin L, Wang M, Liu T, Jiang X, Gao H (2023) Small-molecule drugs of colorectal cancer: Current status and future directions. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 166880

Zalewski A, Snook AE, Waldman SA (2021) Stem cells as therapeutic targets in colorectal cancer. Personalized Med 18:171–183

Zandieh MA, Farahani MH, Rajabi R, Avval ST, Karimi K, Rahmanian P, Razzazan M, Javanshir S, Mirzaei S, Paskeh MDA (2023) Epigenetic regulation of autophagy by non-coding RNAs in gastrointestinal tumors: Biological functions and therapeutic perspectives. Pharmacol Res 187:106582

Zhang B, Liu L (2021) Autophagy is a double–edged sword in the therapy of colorectal cancer. Oncol Lett 21:1–8

Zhang H, Tang J, Li C, Kong J, Wang J, Wu Y, Xu E, Lai M (2015) MiR-22 regulates 5-FU sensitivity by inhibiting autophagy and promoting apoptosis in colorectal cancer cells. Cancer Lett 356:781–790

Zhang J, Wang G, Zhou Y, Chen Y, Ouyang L, Liu B (2018) Mechanisms of autophagy and relevant small-molecule compounds for targeted cancer therapy. Cell Mol Life Sci 75:1803–1826

Zhang J, Duan D, Song ZL, Liu T, Hou Y, Fang J (2021) Small molecules regulating reactive oxygen species homeostasis for cancer therapy. Med Res Rev 41:342–394

Zhao S, Chen S-r, Yang X-f, Shen D-f, Takano Y, Su R-j, Zheng H-c (2017) BTG1 might be employed as a biomarker for carcinogenesis and a target for gene therapy in colorectal cancers. Oncotarget 8:7502

Zheng S, Zhong Y-F, Tan D-M, Xu Y, Chen H-X, Wang D (2019) Mir-183-5p enhances the radioresistance of colorectal cancer by directly targeting ATG5. J Biosci 44:1–11

Zhou M, Niu H, Cui D, Huang G, Li J, Tian H, Xu X, Liang F, Chen R (2024) Resveratrol triggers autophagy-related apoptosis to inhibit the progression of colorectal cancer via inhibition of FOXQ1. Phytother Res 38:3218–3239

Zhu J, Zhao B, Xiong P, Wang C, Zhang J, Tian X, Huang Y (2018) Curcumin induces autophagy via inhibition of yes-associated protein (YAP) in human colon cancer cells. Med Sci Monitor: Int Med J Experimental Clin Res 24:7035

Zhu W, Long J-L, Yin Y-T, Guo H-N, Jiang E-P, Li Y-L, He Q-L, Zeng C, Sun Y-Q (2019) MicroRNA-34a suppresses the invasion and migration of colorectal cancer cells by enhancing EGR1 and inhibiting vimentin. Experimental Therapeutic Med 18:2459–2466