Modulation of Reactive Oxygen Species to Overcome 5-Fluorouracil Resistance

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Abstract
5-Fluorouracil (5-FU) remains to be an important chemotherapeutic drug for treating several cancers when targeted therapy is unavailable. Chemoresistance limits the clinical utility of 5-FU, and new strategies are required to overcome the resistance. Reactive oxygen species (ROS) and antioxidants are balanced differently in both normal and cancer cells. Modulating ROS can be one method of overcoming 5-FU resistance. This review summarizes selected compounds and endogenous cellular targets modulating ROS generation to overcome 5-FU resistance.

Key Words: Reactive oxygen species, Cancer, Resistance, 5-Fluorouracil

INTRODUCTION

Despite the introduction of targeted anticancer therapy, 5-fluorouracil (5-FU) remains an important chemotherapeutic drug for treating several cancers, including colorectal, breast, and gastric cancer. 5-FU’s cytotoxic mechanism involves the inhibition of thymidylate biosynthesis or the misincorporation of fluorinated nucleotides into newly synthesized DNA or RNA (Longley et al., 2003). It can be effective in the treatment of cancer when targeted therapy is unavailable. As described in previous studies, the development of prodrugs such as capecitabine has improved the limitation of 5-FU due to poor oral absorption (Pazdur et al., 1998). Furthermore, combination chemotherapy improved 5-FU’s anticancer effect, as demonstrated by FOLFOX (folinic acid, 5-FU, and oxaliplatin) and FOLFIRI (folinic acid, 5-FU, and irinotecan) (Souglakos et al., 2006). Combining chemotherapeutics with different mechanisms could overcome the heterogeneity of tumor cells and decrease the development of resistance (Frei et al., 1998). Nevertheless, the overall response rate remains less than 50% (Mehrzad et al., 2016) due to the cells being resistant to chemotherapy.

Studies have been conducted to elucidate the 5-FU resistance mechanism described elsewhere (Blondy et al., 2020). The generation of reactive oxygen species (ROS) frequently correlates with the induction of apoptosis in many cancer cells; modulation of ROS may be one mechanism by which cancer cells avoid the cytotoxicity induced by 5-FU (Mates and Sanchez-Jimenez, 2000). For example, in human lung carcinoma cells NCI-H1299, the expression of reactive oxygen modulator 1 (Romo1) is elevated, and the cellular level of ROS is high. At the same time, tumor cells maintain high levels of antioxidant enzymes and antiapoptotic Bcl-2 family proteins, most likely to reduce oxidative stress (Hwang et al., 2007). This implies that cancer cells prefer a high level of ROS while keeping the protective mechanisms running to minimize the unwanted toxicity of ROS.

ROS have a versatile role in cancer cell biology (Liou and Storz, 2010). When elevated, ROS are thought to act as mitogens, inducing cancer cell proliferation (Torres and Forman, 2003). DNA damage from oxidative stress may lead to mutations that can either activate oncogenes or inactivate tumor suppressor genes (Wei, 1992). ROS production is minimal in normal cells, and antioxidant functions effectively remove ROS (Fig. 1A). Increased ROS production is frequently observed in cancer cells with a poor prognosis (Kumar et al., 2008). Cancer cells maintain a relatively high level of ROS, likely due to the tumor-promoting effects of ROS such as angiogenesis (Ushio-Fukai and Nakamura, 2008), metastasis (Nishikawa, 2008), and proliferation (Juhasz et al., 2017). As shown in Fig. 1B, cancer cells increase the level of antioxidant systems in response to elevated levels of ROS to protect...
themselves from oxidative stress (Gorrini et al., 2013). Many anticancer drugs, including 5-FU, induce high levels of ROS to exert cytotoxic effects. Cancer cells adapt to the escalated ROS level by expressing even more antioxidant systems (Fig. 1C) (Liu et al., 2016b). When there is insufficient protection from high levels of ROS, cancer cells may not survive (Fig. 1D).

In this study, we summarized our understanding of natural and synthetic compounds (Table 1) and identified possible cellular targets involved with the modulation of cellular ROS levels to overcome 5-FU resistance.

**Table 1.** Selected compounds increasing ROS generation to overcome 5-FU resistance

| Compound                        | Cell/tissue type       | Effects/Mechanisms                                                                 |
|---------------------------------|------------------------|-----------------------------------------------------------------------------------|
| tetrathiomolybdate              | Ovarian cancer cells   | Stress-mediated apoptosis↑, activation of JNK and p38 MAPK↑                         |
| TPEN                            | Colon cancer HCT116    | Mitochondrial membrane potential (MMP)↓                                             |
| apigenin                        | Hepatocellular carcinoma cells | Mitochondrial apoptosis↑                                                              |
| Polyphenolics from quince       | Colon cancer cells LS174 | NF-κB activation↑, cell cycle progression↓, angiogenesis↓                           |
| kaempferol                      | Colon cancer cells LS174 | Activation of STAT3↓, angiogenesis↓                                               |
| shikonin                        | Gastric cancer SGC-7901 | Translocation of AIF and Endo G into nucleus                                       |
| proanthocyanidin                | Breast cancer MDA-MB-231 cells | G2/M cell cycle arrest↑, MMP↓                                                        |
| B63 (curcumin analog)           | Gastric cancer cells SGC-7901 etc. | Expression of Thiododoxin reductase 1↓                                          |
| dimethoxycurcumin               | Colon cancer cells SW480, SW620 | Expression of Bax and cyt C↑, expression of Bcl-2↓                                |
| Sanguisorba officinalis L. radix | Colonrectal cancer cells RKO, HCT116 | Bax/Bcl-2 disruption↑, autophagy↑                                                   |
| manuka honey                    | Colon cancer cells HCT116 | Expression of EGFR, HER2, Akt and mTOR↓                                           |
| emodin                          | Breast cancer MCF7 cells | Expression of E2F1 and NRPARP↓                                                     |
| gypenoside                      | Colorectal cancer cells SW-480, SW-620 and Caco2 | DNA damage induction↑, expression of p53↑                                         |
| tubeimoside-I                   | Colorectal cancer cells SW480, SW620, HCT116, and RKO | Activation of AMPK↑                                                                 |
| oridonin                        | Colorectal cancer cells HCT115 | Activation of JNK/c-Jun pathway↓                                                   |
| Coptis herb extracts            | Lung cancer A549 cells | ROS↑                                                                              |
| mahanine                        | Colorectal cancer cells HCT116, SW480 | Expression of PTEN and p53 in nucleus↑                                           |
| caffeine                        | Liver cancer cells HepG2, HLF, Huh7, etc. | Cleavage of PARP↑, expression of Bcl-2 and Bcl-xL↓                               |
| selenocysteine                  | Skin cancer cells A375 | Activation of ERK/Akt signaling↓                                                   |
| allicin                         | Liver cancer cells SK-Hep-1, BEL-7402 | ROS↑, MMP↓                                                                     |
| 3-bromopyruvate                 | Liver cancer cells SNU449, Hep3B | ROS↑, MMP↓                                                                       |

**NATURAL/SYNTHETIC COMPOUNDS THAT MODULATE ROS TO OVERCOME 5-FU RESISTANCE**

**Metal chelators**

Tetrathiomolybdate, a copper-chelating drug, was initially developed as an anticopper and antiangiogenic agent to treat Wilson's disease (Brewer et al., 1991). Interestingly, it enhances the activity of the anticancer drug doxorubicin, a DNA intercalator in ovarian cancer cells (Kim et al., 2011). Tetrathiomolybdate increased the cytotoxicity of doxorubicin at subcytotoxic levels, likely by targeting antioxidant enzymes such as...
copper/zinc–superoxide dismutase (SOD). Furthermore, by generating ROS, tetrathiomolybdate increased the cytotoxicity of several anticancer drugs, including 5-FU and mitomycin C (Kim et al., 2012). The production of ROS induced by tetrathiomolybdate resulted in the activation of stress-mediated apoptosis, JNK, and p38 mitogen-activated protein kinase (MAPK), which increased cytotoxicity.

*N,N,N′,N′*-tetrakis-[2-pyridylmethyl]-ethylenediamine (TPEN) was reported to have a cancer-specific copper chelation mediated cytotoxicity (Fatfat et al., 2014). Additionally, TPEN treatment resulted in the excessive generation of ROS via the formation of the TPEN-copper complex, leading to cytotoxicity in human colon cancer HCT116 cells. Evidently, elevated copper levels may be important in maintaining the proper level of ROS generation in cancer cells, whereas intracellular copper levels are crucial to maintaining the proper level of ROS generation in cancer cells (Gupte and Mumper, 2009). Furthermore, although cellular copper levels may be a target for cancer treatment, it remains to be seen whether a copper-chelating drug can help overcome 5-FU resistance.

**Phenolic compounds**

Interestingly, several antioxidant compounds promote the production of ROS in cancer cells. Although more research is needed to elucidate the precise mechanisms, these antioxidant compounds are thought to modulate ROS generation and increase the cytotoxicity of 5-FU. Phenolic compounds refer to diverse natural products such as flavanols, flavonols, chalcones, tannins, curcuminoids, etc. Their antioxidant function is usually attributed to the phenolic ring structure (Cai et al., 2006). The following sections list several phenolic compounds (Fig. 2) that have been reported to have synergistic cytotoxicity when combined with 5-FU or to be cytotoxic to 5-FU resistant cancer cells.

Apigenin is a flavonoid compound found in common fruits and vegetables that exhibits anti-inflammatory, antioxidant, and anticancer activity (Shukla and Gupta, 2010). Research revealed that apigenin cotreatment with 5-FU at a subtoxic level demonstrated synergistic cytotoxicity in treating hepatocellular carcinoma (HCC) cells in vitro and in vivo (Hu et al., 2015). Moreover, the ROS level was increased, and the mitochondrial apoptotic pathway was activated, indicating that apigenin has a pro-oxidant function. Although it remains to be seen whether apigenin is cytotoxic to 5-FU resistant cancer cells, apigenin, which is well-known for its antioxidant activity, appears to also demonstrate some pro-oxidant activity.

**Fig. 2.** Phenolic compounds.
The polyphenolic extract from quince (Cydonia oblonga Miller) has shown antiproliferative effects in kidney and colon cancer cells (Carvalho et al., 2010). A Tunisian research group reported that quince peel polyphenolic extract induced ROS production, and the cytotoxic effect of 5-FU was increased in human colon adenocarcinoma LS174 cell (Riahi-Chebbi et al., 2015). Although the potential expansion of the cellular work to a preclinical level requires further study, it is worth noting that ROS generation may be linked to the cytotoxicity of 5-FU. Riahi-Chebbi et al. (2019), conversely, reported that kaempferol, another phenolic compound derived from quince, inhibited the production of ROS while exhibiting the same cytotoxicity as other phenolic compounds and was effective even in 5-FU resistant colon cancer cells. This intriguing result cautions us not to assume that a decrease in ROS levels is cytoprotective, as other mechanisms may simultaneously be responsible for cytotoxicity.

Shikonin, a naphthoquinone derivative found in the shikonin plant (Lithospermum erythrohizon), is known for its cytotoxicity and anti-inflammatory activity (Chen et al., 2002). Similarly, Liang et al. (2016) studied the antitumor activity of shikonin on gastric cancer. They observed that shikonin induced ROS generation and enhanced the 5-FU sensitivity in vitro and in vivo. In addition to the mitochondria-mediated apoptotic pathway, they detected the caspase-independent nuclear translocation of the apoptosis-inducing factor and endonuclease G from mitochondria.

Proanthocyanidin compounds from white fig Ficus virens (Chen et al., 2017b) and Uncaria rhynchophylla (Chen et al., 2017c) have been shown to have cytotoxic activity on human breast cancer MDA-MB-231 cells. Proanthocyanidins increased cellular ROS and the mitochondrial apoptotic pathway, and synergistic cytotoxicity was observed when proanthocyanidins were combined with 5-FU. Surprisingly, the cytotoxic effect appeared to be cancer cell-specific, and proanthocyanidins alleviated intestinal mucositis in 5-FU-treated rats (Chen et al., 2017b).

Curcumin, a polyphenolic compound frequently found in curry powders, has long been considered an antioxidant (Ak and Gulcin, 2008). Several studies, however, have reported the generation of ROS by curcumin analogs. Researchers created B63, a curcumin analog, as an anticancer agent and discovered that B63 induced ROS-mediated apoptosis in gastric cancer cells (Chen et al., 2019). They showed the inhibition of thioredoxin reductase 1 (TrxR1) by B63 in vitro, and the overexpression of TrxR1 negated the proapoptotic activity of B63. Their findings indicate that TrxR1 is a target of B63 and that B63 effectively suppressed the growth of 5-FU-resistant gastric cancer cells. Similarly, dimethoxycurcumin increases ROS production in colon cancer cells, allowing it to exert cytotoxic activity against colon cancer SW480 and SW620 cells when combined with 5-FU (Zhao et al., 2017).

A Chinese research group studied the water extract of Sanguisorba officinalis L. radix, for its anticancer activity on human colorectal cancer HCT116 and RKO cells (Liu et al., 2016a). They demonstrated that treating cells with the extract and 5-FU significantly increased ROS generation and that cotreatment increased 5-FU cytotoxicity. Moreover, they reported an increased in autophagy-related markers, light chain LC3, and p62, besides ROS generation, implying that the generation of ROS is not the only explanation for the synergism between Sanguisorba officinalis L. radix and 5-FU. The study demonstrated that gallic acid, catechinic acid, and ellagic acid, three main constituents of Sanguisorba officinalis L. radix, are responsible for the synergistic activity.

Manuka honey, a type of honey collected from the manuka tree Leptospermum scoparium, has antioxidant, anti-inflammatory, and anticancer properties (Afrin et al., 2018b). Reports describe the synergistic cytotoxicity of manuka honey on human colon cancer HCT116 and LoVo cells when combined with 5-FU (Afrin et al., 2018a). Manuka honey, a polyphenol-rich natural product, suppressed cell survival signals in HCT116 and LoVo cells while inducing pro-apoptotic signals and ROS production. Furthermore, the combined treatment reduced the activity of antioxidant enzymes such as SOD, catalase, glutathione peroxidase, glutathione reductase, and the expression of Nrf2, SOD, catalase, and HO-1, resulting in increased cell death due to oxidative stress.

Emodin, a natural anthraquinone compound, has antiproliferative activity in human breast cancer MCF7 cells (Huang et al., 2007). In a later study, tests were conducted to determine whether low-dose emodin could potentiate the activity of 5-FU in MCF7 cells (Zu et al., 2018). Findings revealed that emodin increased 5-FU-induced apoptosis in breast cancer cells by generating ROS. Surprisingly, researchers observed cellular senescence after 5-FU treatment with emodin, which they believe was caused by the upregulation of cyclin-dependent kinase inhibitors and the downregulation of EZF1 and the notch-regulated ankyrin repeat protein (NRARP) protein. Their findings suggested that NRARP is a critical target for inducing cellular senescence.

Polycyclic compounds and alkaloids

Several polycyclic compounds and alkaloids (Fig. 3) have been investigated for their role in producing ROS in cancer cells. For instance, gypenosides are triterpenoid saponin compounds whose potential use in cancer treatment has been documented (Ahmad et al., 2019), and they are thought to have potentiated 5-FU’s cytotoxicity (Kong et al., 2015). Results showed that p53 and ROS generation mediates the synergism between gypenosides and 5-FU to exert anticancer activity. Additionally, the triterpenoid saponin compound, tubeimoside-I, isolated from Rhizoma Bolbostemmatum, has exhibited antitumor activity in various types of tumors (Yu et al., 1994). Yan et al. (2019) discovered that combining 5-FU and tubeimoside-I suppressed the growth of colorectal cancer SW480, SW620, HCT116, and RKO cells in a synergistic manner, whereas tubeimoside-I induced cellular ROS and the activation of AMPK, resulting in cytotoxic autophagy.

Oridonin, a diterpenoid from the medicinal herb Rabdosia rubescens, exhibits antitumor activity (Li et al., 2011). Studies assessed oridonin’s anticancer effect in colorectal cancer HCT15 cells and compared the 5-FU resistant HCT15 cells and sensitive cells (Zhang et al., 2019). To exert its cytotoxicity, oridonin induced the generation of ROS in both cells and the activation of the JNK/c-Jun pathway. Notably, cotreatment with N-acetylcysteine reversed JNK/c-Jun pathway activation, indicating that ROS generation mediates JNK/c-Jun pathway activation. Although oridonin activated apoptosis in colorectal cancer cells, it appears to activate necroptosis in renal carcinoma 786-O cells (Zheng et al., 2018). Cotreatment of oridonin and 5-FU showed synergistic cytotoxicity, probably through separate mechanisms, and notably, the same compound showed a different mechanism of action.
The anticancer effects of the *Coptis* herb extracts and the major alkaloid component berberine have been well-reported, and their cytotoxic effects have been detected in various cancer cell lines (Tang *et al*., 2009). Furthermore, *Coptis* extract showed cytotoxicity when combined with 5-FU in human lung cancer A549 cells (He *et al*., 2012). The cytotoxicity of either *Coptis* extract or berberine was associated with an increase in ROS generation in a dose-dependent manner, and when combined with 5-FU, the anticancer effect was enhanced.

Mahanine, an alkaloid from the curry leaf plant (*Murraya koenigii*), has exhibited various biological activities (Ramsewak *et al*., 1999). Das *et al*. (2014) showed the synergistic enhancement of cytotoxicity of 5-FU when mahanine was used together in human colorectal cancer HCT116 and SW480 cells. Interestingly, the synergistic effect was observed irrespective of p53 status, i.e., both p53wt and p53null cells were sensitive to mahanine in combination with 5-FU. Although the precise mechanism is unknown, mahanine induced ROS production and led to the accumulation of PTEN and p53 in the nucleus. The increased production of ROS appears to be linked to the activation of tumor suppressor proteins PTEN and p53, resulting in increased cytotoxicity of 5-FU.

Caffeine, a food ingredient found in coffee and tea, slows the growth of liver cancer cells, including HepG2, HLF, Huh7, and PLC/PRF/5 (Okano *et al*., 2008). Many studies report a synergistic effect of caffeine and cisplatin in various cancers, such as the human endometrial cancer cell line RL95-2 (Lin *et al*., 2021). Recently, Wang *et al*. (2019) reported that the antitumor activity of 5-FU was enhanced by cotreatment of caffeine in HCC HepG3 and SMMC cells. They discovered that combining 5-FU and caffeine inhibited HCC cell growth and induced apoptosis by increasing ROS production.

**Role of other small molecules in ROS production**

As described below, reports suggest that other small molecules (Fig. 4) may modulate ROS generation in cancer cells. First, selenocystine is the oxidation product of selenocysteine, which has a diselenide bond connecting two amino acids. It induces apoptosis in human cancer cells such as A375, HepG2, and MCF7 by increasing ROS production (Chen and Wong, 2009). Fan *et al*. (2013) investigated whether selenocystine cotreatment could increase the cytotoxicity of 5-FU in human melanoma A375 cells. They observed significant selenocystine-induced DNA damage mediated by ROS production and the inactivation of the extracellular-signal-regulated kinase (ERK) and Akt signaling pathways, resulting in anticancer synergism. Furthermore, the induction of ROS-mediated apoptosis in melanoma cells by 3,3′-diselenodipropionic acid, a selenocysteine derivative, is another example of potentially overcoming anticancer drug resistance (Cao *et al*., 2014).

Allicin, a compound in garlic, has drawn considerable attention as an antimicrobial antioxidant (Ghan *et al*., 2013). Zou Fig. 3. Polycyclic compounds and alkaloids.
et al. (2016) tested whether the anticancer activity of 5-FU in human HCC SK-Hep-1 and BEL-7402 cells and in nude mice increased with allicin and 5-FU cotreatment. They discovered that cotreatment with allicin increased ROS production and sensitization of HCC cells to 5-FU. The synergistic effect was reversed by N-acetylcysteine treatment, indicating that the anticancer activity is mediated by ROS generation. Their study also demonstrated that cotreatment with allicin and 5-FU significantly inhibited the growth of HCC xenograft tumors in nude mice; although commonly thought to be an antioxidant, allicin increased ROS generation when combined with combined 5-FU.

3-Bromopyruvate is an inhibitor of hexokinase (Ko et al., 2001), the key enzyme of glycolysis. The researchers reported that 3-bromopyruvate induced the ROS-mediated cell death of hepatoma SNU449 and Hep3B cells (Kim et al., 2008). Upon treatment with 3-bromopyruvate, both cell lines underwent necrosis and apoptosis in an ATP depletion-dependent manner due to increased intracellular ROS and the disruption of mitochondrial function. Furthermore, the combination of 3-bromopyruvate and 5-FU inhibited tumor growth in vivo and in vitro (Chong et al., 2017).

![Chemical structures of selenocysteine, N-acetylcysteine, allicin, 3-bromopyruvate, and ubenimex.](https://doi.org/10.4062/biomolther.2022.017)

**Fig. 4.** Other small molecules.

### ENDOGENOUS CELLULAR TARGETS TO OVERCOME 5-FU RESISTANCE

**Nuclear factor erythroid 2-related factor 2 (Nrf2)**

The transcription factor Nrf2 mediates antioxidant response (Moi et al., 1994). Nrf2 exists in the cytoplasm as the Nrf2-Keap1 complex in the absence of oxidative stress. The cellular level of Nrf2 is kept low by continuous degradation via the ubiquitin–proteasome system, which is mediated by Keap1, the Nrf2 key repressor (Zhang, 2006). Several cysteine residues of Keap1 are modified when exposed to oxidative stress, resulting in the dissociation of the Nrf2-Keap1 complex. Nrf2, which is released by Keap1, enters the nucleus and binds to the DNA in the antioxidant response element (ARE) region to regulate the expression of several genes involved in antioxidant function, such as glutamate-cysteine ligase catalytic subunit (Solis et al., 2002), thioredoxin reductase (Soriano et al., 2009), and heme oxygenase-1 (HO-1) (Jarmi and Agarwal, 2009). When expressed, these antioxidants may impart some degree of protection to cells under oxidative stress. Overexpression of Nrf2 in gastric cancer serves as a prognostic marker for 5-FU resistance, lending credence to Nrf2’s prosurvival role (Hu et al., 2013). Similarly, Nrf2 has a role in developing 5-FU resistance in colon cancer HT-29 cells (Akhdar et al., 2009). Kang et al. (2014) discovered hypomethylation of Nrf2 promoter CpG islands in 5-FU resistance colorectal cancer SNU5/5-FUR cells compared with nonresistant cancer cells, indicating that Nrf2 upregulation led to 5-FU resistance. Besides its antioxidant function, Nrf2 regulates the expression of drug-metabolizing enzymes and drug transporters, resulting in a decrease in 5-FU efficacy (Bai et al., 2016). A team of researchers reported that 2’,4’-dihydroxy-6’-methoxy-3’,5’-dimethylchalcone, an inhibitor of Nrf2/ARE pathway, could reverse 5-FU resistance in HCC BEL-7402 cells by inhibiting the 5-FU efflux (Wei et al., 2018).

### ROS/mitogen-activated protein kinases pathway

JNK, c-Jun N-terminal kinase, belongs to MAPKs. The function of JNK is related to both cell survival (Wu et al., 2019) and death (Dhanasekaran and Reddy, 2008). Based on the stimuli, JNK signaling can be either prosurvival or pro-apoptotic, and the signaling pathway is not directly linked to the cytotoxic effect of 5-FU. It appears that either activation or inactivation of the proper signaling pathway could place an additional burden on cells treated with 5-FU, potentially increasing 5-FU cytotoxicity.

Compared with differentiated and chemosensitive pancreatic cancer stem cells, the JNK signaling pathway is activated in pancreatic cancer stem cells (Okada et al., 2014; Suzuki et al., 2015). Researchers established that the JNK signaling pathway is activated in the pancreatic cancer stem cells (Suzuki et al., 2015). Pretreatment of cells with SP600125, a JNK inhibitor, resulted in the sensitization of the cells to 5-FU and gemcitabine. The cytotoxic effects of these chemotherapeutics were accompanied by an increase in ROS production. Furthermore, the use of N-acetylcysteine, a free radical scavenger, reduced the intracellular level of ROS and allowed the cells to remain resistant to 5-FU; this is an example of the detrimental use of an antioxidant in chemotherapy. The synergistic cytotoxicity of 5-FU and the compounds mentioned above, tetramethylomolybdate (Kim et al., 2012) and oridonin (Zhang et al., 2019), is associated with the generation of ROS and the
activation of JNK. 5-FU cytotoxicity appears to be enhanced by oxidative stress and JNK activation, potentially overcoming activation of JNK. 5-FU cytotoxicity appears to be enhanced in cancer SKBR3 MDA-MB-231 cells. (2017b) reported that 5-FU resistant cancer cells were affected. Initially, the prosurvival role of autophagy was reported because inhibition of autophagy was associated with increased cytotoxicity of 5-FU in human colon cancer colon26 and HT29 cells in vitro and in vivo (Li et al., 2010). The prosurvival role of autophagy was again shown in HCT116 p53−/− cells (Sui et al., 2014) and human HCC Bel-7402 cells (Wang et al., 2017a). The involvement of prosurvival autophagy in 5-FU resistance has been demonstrated for several cellular components such as TSPAN9 (Qi et al., 2020) and claudin-1 (Tong et al., 2019). Zhang et al. (2017) reported synergistic cytotoxicity from the combination of curcumin and 5-FU, which included a reduction in prosurvival autophagy mediated by AMPK and Unc-51 Like Autophagy Activating Kinase 1 (ULK1). By contrast, several compounds have been shown to increase the cytotoxicity of 5-FU by inducing prodeath autophagy: β-Elemene, a sesquiterpene compound found in various plants, induces prodeath autophagy in 5-FU resistant colorectal cancer HCT116 p53−/− cells (Zhang et al., 2020). Similarly, when combined with 5-FU, withaferin-A, a natural product with a steroidal lactone structure, induces endoplasmic reticulum stress-mediated autophagy (Alnuqaydan et al., 2020) in CRC cells (SW480, HT29, HCT116).

Aminopeptidase N (CD13)

Aminopeptidase N, also known as CD13, is a cell-surface-anchored zinc peptidase with various functions, including peptide cleavage, endocytosis, and signaling (Mina-Osorio, 2008). It was initially identified as a cell surface marker CD13 for myeloid leukemia cells (Sakai et al., 1987), and the signaling function of aminopeptidase N appears to be independent of enzyme activity. Aminopeptidase expression has been linked to a poor prognosis and angiogenesis in cancer cells such as nonsmall cell lung cancer (Tokuhara et al., 2006), pancreatic carcinoma (Ikeda et al., 2003), and colon cancer (Hashida et al., 2002). When substrates or inhibitors bind to CD13, the conformation of the dimeric CD13 structure changes, resulting in signal transduction (Xu et al., 1997), and CD13 protects cells from apoptosis by reducing ROS-induced DNA damage.

Uibenimex, also known as bestatin, is a dipeptide compound produced by actinomycetes (Umezawa et al., 1976). It specifically blocks and antagonizes CD13 for myeloid leukemia cells (Sakai et al., 1987), and the signaling function of aminopeptidase N appears to be independent of enzyme activity. Aminopeptidase expression has been linked to a poor prognosis and angiogenesis in cancer cells such as nonsmall cell lung cancer (Tokuhara et al., 2006), pancreatic carcinoma (Ikeda et al., 2003), and colon cancer (Hashida et al., 2002). When substrates or inhibitors bind to CD13, the conformation of the dimeric CD13 structure changes, resulting in signal transduction (Xu et al., 1997), and CD13 protects cells from apoptosis by reducing ROS-induced DNA damage.

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CONCLUSION

In this study, we have compiled a list of compounds that can be used alone or combined with 5-FU to modulate ROS generation. Although the precise mechanisms underlying their cytotoxicity remain unknown, several endogenous cellular tar-
gets have been identified, as described above. These compounds and cellular targets could help develop new strategies for combating 5-FU resistance.

CONFLICT OF INTEREST

The authors claim no conflicts of interest.

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