5-Fluorouracil (5-FU) is among the most administrated chemotherapeutic agents for a wide variety of neoplasms. Non-coding RNAs have a central impact on the determination of the response of patients to 5-FU. These transcripts via modulation of cancer-related pathways, cell apoptosis, autophagy, epithelial–mesenchymal transition, and other aspects of cell behavior can affect cell response to 5-FU. Modulation of expression levels of microRNAs or long non-coding RNAs may be a suitable approach to sensitize tumor cells to 5-FU treatment via modulating multiple biological signaling pathways such as Hippo/YAP, Wnt/β-catenin, Hedgehog, NF-kB, and Notch cascades. Moreover, there is an increasing interest in targeting these transcripts in various kinds of cancers that are treated by 5-FU. In the present article, we provide a review of the function of non-coding transcripts in the modulation of response of neoplastic cells to 5-FU.

Keywords: lncRNA, miRNA, fluorouracil, expression, biomarker
INTRODUCTION

5-fluorouracil (5-FU) and its oral prodrugs including S1 and capecitabine (1) are among the main components of most chemotherapeutic regimens whose efficiencies have been established in the treatment of several neoplasms such as head and neck squamous cell carcinoma (SCC) (2), gastrointestinal SCC and adenocarcinoma (ADC) (3, 4), and SCC of the uterine cervix (5). This agent was introduced by Heidelberger et al. during the 1950s (6). Afterward, it has been increasingly used during the last decades and remained as the backbone of most of chemotherapeutic regimens. 5-FU has also been used in combination with novel cancer therapies especially targeted therapeutics including vascular endothelial growth factor (VEGF) inhibitors [bevacizumab (7), ziv-afibercept (8), regorafenib (9) and ramucirumab (10)] and anti-epidermal growth factor receptor (EGFR) therapies [cetuximab (11) and panitumumab (12)]. The cytotoxic effect of 5-FU is mainly considered as the main mechanisms of resistance. Focaccetti et al. (24) showed clear signs of autophagy along with the significant increase in apoptosis in endothelial cells and cardiomyocytes after treatment with 5-FU. Interestingly, more recently autophagy is proposed as a potential way of acquired resistance of tumoral cells towards 5-FU (25). Globally, the alternation of TS coding gene, i.e. TYMS (26), single nucleotide polymorphisms affecting the activity of methylenetetrahydrofolate reductase (MTHFR) (27), and Dihydropyridine dehydrogenase (DPD) expression (28) are considered as the main mechanisms of resistance.

CATABOLIC AND ANABOLIC WAY OF 5-FLUOROURACIL TRANSFORMATION

5-FU is a uracil analog with a fluorine atom at the C-5 position. After intravenous administration of 5-FU, this medication can rapidly enter target cells using the same transport mechanism as uracil (15). Accumulating evidence demonstrated that the transportation of 5-FU could be passively triggered via transcellular and paracellular pathways in tumor cell monolayers. In addition, 5-FU by passive diffusion can promptly pass the blood-brain barrier (BBB) (18, 29). 5-FU can be transformed to the following active metabolites in the target cells: 1) Fluorodeoxyuridine triphosphate (FdUTP) that could combine into DNA rather than deoxythymidine triphosphate (dTTP); 2) Fluorouridine triphosphate (FUTP) that could combine into RNA rather than uridine triphosphate (UTP). FUTP alters RNA function and processing, and FdUTP damages DNA directly by misincorporation into it. In other hand, FUTP is incorporated into RNA resulting in substantial damage in this molecule. Finally, 5-FU causes cell death through simultaneous induction of apoptosis and autophagy (19, 20). Xiong et al. (19) showed that treatment of Bax or PUMA deficient human colon cancer cells with 5-FU resulted in reduction of mTOR activity and subsequent up-regulation of autophagy in this cell line resulting in considerable inhibition of cell proliferation. Moreover, Yang et al. (21) have shown that treatment of human gastric cancer cells with 5-FU resulted in inhibition of cell proliferation through autophagic process resulting from 5-FU-related miR-30 suppression and Beclin-1 upregulation. Besides, mTOR activity and miR-30 suppression as potential pathways for 5-FU-related autophagy activation, Cottone et al. (22) showed that 5-FU can stimulate the inflammatory cells which are responsible for High Mobility Group Box 1 (HMGB1) release resulting in the exacerbation of the autophagy activation. In this context, Nyhan et al. (23) used HMGB1 as a marker of non-apoptotic cell death showing the increase of autophagy after the treatment of human oesophageal cancer cell lines with 5-FU in the presence of miR-193b overexpression. In addition to tumoral cells, non-malignant cells are also influenced by 5-FU-related autophagy. Focaccetti et al. (24) showed clear signs of autophagy along with the significant increase in apoptosis in endothelial cells and cardiomyocytes after treatment with 5-FU. Interestingly, more recently autophagy is proposed as a potential way of acquired resistance of tumoral cells towards 5-FU (25).
diphosphate (FUDP) that is either subsequently phosphorylated to the active metabolite FUTP or could be transformed to fluorodeoxyuridine diphosphate (FdUDP) via ribonucleotide reductase (16). Afterward, FdUDP is either phosphorylated to FdUTP or could be dephosphorylated to FdUMP. Therefore, both dUTP and FdUMP could effectively contribute to DNA damage. The transformation of 5-FU to FdUMP in the alimentary canal and bone marrow could in turn lead to digestive tract toxicity as well as myelotoxicity (1). Additionally, dihydropyrimidine dehydrogenase (DPYD), which is a ubiquitous and rate-limiting enzyme of the uracil catabolic pathway and is present in the liver, gut, intestinal mucosa, and several other tissues, could be considered as a major enzyme for the degradation of 5-FU (1, 31). 5-FU represents poor bioavailability because of its prompt catabolic degradation to 5, 6-dihydro-5-fluorouracil (DHFU) via DPYD (25). DPYD could catabolize 5-FU to 5, 6-dihydro-5-fluorouracil (DHFU), eventually resulting in the creation of α-fluoro-β-ureidopropionic acid (FUPA) as well as α-fluoro-β-alanine (FBAL) that subsequently could be excreted through the kidneys (32). Oral administration of 5-FU in the form of 5-FU pro-drugs (oral FPs) could result in imperfect and disordered bioavailability because of variation in the function of DPYD. Hence, it is correlated to unpredictable levels of 5-FU in the plasma because of noticeable intra- and inter-patient mutability in its adsorption/deletion (33).

NON-CODING RNAS AND RESPONSE TO 5-FU

Non-coding RNAs modulate several cellular pathways that are involved in the response of neoplastic cells to 5-FU or its oral prodrugs (34). In a broad classification, we categorize non-coding RNAs to long non-coding RNAs (IncRNAs) and microRNAs (miRNAs) with sizes more than 200 nucleotides and about 22 nucleotides, respectively. These two main classes of non-coding RNAs vary in their mode of action in the regulation of gene expression, however, both regulate fundamental aspects of cellular activities among them are apoptosis, autophagy, and DNA repair (35, 36). These cellular processes define the response of neoplastic cells to chemotherapeutic agents such as 5-FU.

miRNAS AND 5-FU RESPONSE

The impact of miRNAs in the modulation of 5-FU resistance has been largely assessed in colorectal cancer (CRC) cells. For instance, as a tumor suppressor miRNA, expression of miR-15b-5p has been down-regulated in tissues and cells obtained from patients with this kind of neoplasm. Up-regulation of miR-15b-5p has enhanced 5-FU-associated cell apoptosis and ameliorated cell response to 5-FU both in vitro and in animal models. NF-κB signaling pathway has been identified as the mediator of miR-15b-5p effect on response to 5-FU. This miRNA negatively regulates NF-κB1 and IKK-α. mir-15b-5p has also been shown to target the anti-apoptotic gene XIAP (37). In CRC cells, miR-21 influences response to 5-FU through targeting PDCD4 and hMSH2 (38, 39). Notably, PDCD4 has also been shown to be targeted by miR-1260b. This miRNA confers resistance to 5-FU and inhibits apoptosis in CRC cells via the PI3K/Akt signaling pathway (40). Expression of miR-21 has been considerably increased in exosomes of CRC cells versus normal human colon epithelium. Exosomal miR-21 can enhance the expression of genes participating in cell proliferation, invasiveness, and extracellular matrix construction. Moreover, miR-21 through targeting PDCD4 can increase resistance to 5-FU (38). Another experiment in this kind of cancer has shown the role of miR-22 in the enhancement of sensitivity to 5-FU through inhibition of autophagy and boosting apoptosis. These effects of miR-22 are mediated through the suppression of expression of B-cell translocation gene 1 (BTG1) (41). Treatment of CRC cells with 5-FU has resulted in enhancement of miR-23a while down-regulation of APAF-1 in these cells. miR-23a antisense has enhanced the activation of the caspases-3 and -7 through up-regulation of miR-23a target APAF-1 and increased the 5-FU-associated apoptosis. Yet, miR-23a antagonism did not surge the anticancer impact of 5-FU in the xenograft model of CRC (42). The tumor-suppressive miRNA miR-199b directly targets the PP2A inhibitor SET, a crucial factor in conferring resistance to 5-FU. An experiment in rectal cancer cells has shown that both miR-199b up-regulation and SET suppression can combat 5-FU resistance. Expression of miR-199b has been decreased in about one-fourth of cases in association with lymph node positivity following chemoradiotherapy and advanced stage (43). Table 1 summarizes the influence of miRNAs on response of CRC cells to 5-FU.

Hepatocellular carcinoma (HCC) is another type of cancer in which the role of miRNAs in the regulation of response to 5-FU has been vastly assessed. Forced over-expression of miR-122 in hepatoblastoma cells has reduced expressions of Bcl-2 and Bcl-XL while increasing P53 protein levels. These effects have been accompanied by enhancement of apoptosis and higher sensitivity to 5-FU, demonstrating the impact of this miRNA in response to 5-FU (86). Another experiment in several HCC cell lines has shown lower expression of miR-125b in 5-FU-resistant cells compared with sensitive cells. Transfection of pre-miR-125b into HCC cells has led to the improvement of sensitivity to 5-FU. F-FU resistant cells also exhibited higher glucose uptake and lactate synthesis compared with 5-FU-sensitive cells. Remarkably, miR-125 has been shown to decrease glucose metabolism by influencing the expression of hexokinase II (87). miR-147 is a tumor suppressor miRNA whose expression has been reduced in HCC cell lines and clinical samples. Overexpression of miR-147 has suppressed in vitro proliferation and migration of HCC cells and enhanced cytotoxic effects of 5-FU. Moreover, it has decreased in vivo tumorigenicity of HCC cells. HOXC6 has been recognized as the downstream target of miR-147 through which this miRNA enhances 5-FU sensitivity (88). Another tumor suppressor miRNA in HCC namely miR-503 regulates the expression of EIF4E and enhances response to 5-FU (89). Table 2 demonstrates the impact of miRNAs modulation of response to 5-FU in HCC cells.
| microRNA | Animal/Human | Cell line | Targets/Regulators | Function | Ref |
|---------|-------------|-----------|-------------------|----------|-----|
| miR-15  | 62 pairs of CRC and ANTs | HCT116 | Bcl-2, Bcl-XL, NF-kB | MR-15 could sensitize CRC cells to 5-FU and increase apoptosis via NF-κB. | (37) |
| miR-21  | – | HT29, T84, LS174, CRL1831 | PDCD4, TPM1, PTEN, hMSH2, TP, DPD | MR-21 by targeting PDCD4 could promote proliferation, invasion and therapy resistance. | (38) |
| miR-21  | – | HT-29, HT-29/5-FU | hMSH2 | MR-21 by targeting hMSH2 could increase cell proliferation and chemoresistance and inhibit apoptosis. | (39) |
| miR-21  | mouse | Colo-320DM, SW620, HCT-116, SW480, RKO | BTG1 | MR-21 by targeting BTG1 could increase 5-FU sensitivity via inhibiting autophagy and promoting apoptosis in CRC cells. | (44) |
| miR-22  | mouse/human; 94 pairs of CRC and ANTs | SW620, RKO | APAF-1, Caspase-9, DND1 | MR-23a by targeting the APAF-1/Caspase-9 axis could enhance 5-FU resistance in CRC cells. | (42) |
| miR-23a | mouse | HCT116, HT29 | − | − | − |
| miR-24  | – | HCT116, RKO, SW480, SW48, CDD-180, HT-29, LOVO, HT-29/5-FU, LOVO/5-FU, FHC | Pgp | MR-26b by targeting Pgp could enhance chemosensitivity to 5-FU. | (45) |
| miR-29c-3p | – | HCT116 p53+/-, HCT116 p53−/- | PHLD22 | MR-29c-3p by targeting PHLD22 could suppress colon cancer cell invasion and migration. | (47) |
| miR-30-5p | 30 pairs of CRC and ANTs | Caco2, HT29, HT15, HCT116, SW620, SW480, 293T | USP22, Wnt/b-catenin | MR-30-5p by targeting USP22 could suppress cell chemoresistance and stemness in CRC cells through the Wnt/b-catenin signaling pathway. | (48) |
| miR-31  | mouse/human; 112 pairs of CRC and ANTs | DLD-1, SW480, WiDr, HT-29, SW48, DLD/F, SW/F, DLD-1, DLD-1/5FU | Fih-1 | − | − |
| miR-34a | – | − | Sirt1, E2F3, P3K/Akt | MR-34a targets Sirt1 and E2F3 genes and decreases resistance to 5-FU. | (49) |
| miR-34a | mouse | SW480, LoVo | Dlil1, Notch | − | − |
| miR-122 | mouse | HCT116/R, HT-9/R, HCT-116, HT-29 | Pkm2 | MR-122 by inhibiting Pkm2 could reverse chemoresistance for 5-FU in CRC cells. | (51) |
| miR-129 | mouse/human; 77 pairs of CRC and ANTs | HCT116, RKO, SW480 | Bcl-2, E2F3, Ts | MR-129 by targeting Bcl-2 could promote apoptosis, inhibit cell proliferation, cause cell-cycle arrest, and also increase response to 5-FU in CRC cells. | (53) |
| miR-133b | – | HT29, HCT116, SW620, 293T | DOT1L | MR-133b by targeting DOT1L could suppress CRC cell stemness and chemoresistance. | (54) |
| miR-133b-182 | mouse/human; 31 pairs of CRC and ANTs | HCT-8, LoVo, HCT-8/5-FU, LoVo/5-FU | ST6GALNAC2, P3K/Akt | MR-135b and miR-82 by targeting ST6GALNAC2 could promote chemoresistance of CRC cells via the P3K/Akt signaling pathway. | (55) |
| miR-139-5p | mouse/human; 204 CRC tissues and 54 normal healthy controls | HT29, LS174T, SW480, SW620, RKO, HCT116, COLO205, LoVo, NCM460 | − | − | − |
| miR-139-5p | – | HCT116, LoVo, HCT-8, HCT-116/5-FU, HCT-116/5-FU | NOTCH-1 | MR-139-5p by targeting NOTCH-1 could sensitize CRC cells to 5-FU. | (57) |
| miR-143 | – | − | − | − | − |
| miR-145 | mouse/human; 152 pairs of CRC and ANTs | SW620, 5-FuR SW620 | − | − | − |
| miR-149 | mouse/human; 152 pairs of CRC and ANTs | HCT-8, LoVo, HCT-8/5-FU, LoVo/5-FU | − | − | − |
| miR-158-3p | 120 pairs of CRC and ANTs | HCT-116, HCT-116, HCT-8, HCT-116/5-FU, HCT-116/5-FU | − | − | − |
| miR-195 | − | HCT-116 | − | − | − |
| miR-196-5p | 15 pairs of CRC and ANTs | Caco-2, HCT8, HCT116, HCT116, SW480 | − | − | − |
| miR-200c | – | HCT-116 | − | − | − |
| miR-203 | mouse | FHG, HCT-116, Caco2, SW480, LoVo/5-FU | − | − | − |
| miR-204 | 33 pairs of CRC and ANTs | LoVo, HT29, SW620, SW116, HCT116, SW480, HcoEpC | − | − | − |
| miR-206 | mouse | HCT116, RKO, HCT116/FR, RKO/FR | Bcl-2 | MR-206b by targeting Bcl-2 could decrease 5-FU resistance in colon cancer cells. | (67) |

| Table 1 | Role of miRNAs in the modulation of response to 5-FU in colorectal cancer (ANT, adjacent normal tissue). | 5FU and Non-Coding RNAs | 3FU and Non-Coding RNAs |
|---------|--------------------------------|------------------|------------------|
| miR-206 | HCT116, RKO, HCT116/FR, RKO/FR | Bcl-2 | MR-206b by targeting Bcl-2 could decrease 5-FU resistance in colon cancer cells. | (67) |
In gastric cancer, miR-31 enhances sensitivity to 5-FU, decreases migration and invasion capacity, and surges the fraction of cells in G1/pre-G1 phase. These effects are possibly mediated through down-regulation of E2F6 and SMUG1 genes (97). On the other hand, miR-147 is an oncogenic miRNA in gastric cancer cells whose silencing has reduced cell proliferation and improved sensitivity of these cells 5-FU via modulating apoptotic pathway. Mechanistically, miR-147 down-regulates the expression of PTEN in gastric cancer cells and consequently modulates PI3K/AKT signaling pathway (98). Besides, the expression of miR-149 has been shown to be elevated in 5-FU-resistant gastric cancer cells compared with the parental cells. Transfection of resistant cells with miR-195 has led to inhibition of HMGA1 expression and down-regulation of miR-29c in tumors and sera of these patients. Figure 1 demonstrates the role of several miRNAs in regulating the sensitivity of cancer cells to 5-FU via modulating the Wnt-β-catenin pathway which is a highly conserved cascade and is activated in the development of various human cancers like colorectal cancer.

Table 3 provides a summary of experiments that reported the impact of miRNAs in the response of gastric cancer to 5-FU. A comprehensive study in esophageal cancer has frequent down-regulation of miR-29c in tumors and sera of these patients. Functionally, miR-29c has been shown to reverse 5-FU resistance in vitro and in vivo through direct interaction with the 3'UTR of...
miR-122
miR-125b
miR-133a/-326
miR-141
miR-145
miR-147
miR-193a-3p
miR-195
miR-200a-3p
miR-302b
miR-503

Table 2: Role of miRNAs in the modulation of response to 5-FU in hepatocellular carcinoma (ANT, adjacent normal tissue).

| microRNA | Animal/Human | Cell line | Targets/Regulators | Function | Ref |
|----------|--------------|-----------|--------------------|----------|-----|
| miR-122  | –            | BEL-7402, BEL-7402/5-FU | Bcl-2, Bcl-XL, p53 | MR-122 by downregulating Bcl-2 and Bcl-XL and increasing p53 could enhance HCC cells sensitivity to 5-FU and induce cell death. | (85) |
| miR-125b | –            | SMMC-7221, Huh7, MHCC-97L, HepG2, HepG2, BEL-7402, THLE-2, THLE-3 | HK II, glycolysis | MR-125b by targeting HK II could sensitize HCC cells to 5-fluorouracil through inhibition of glycolysis. | (87) |
| miR-133a/-326 | – | HepG2 | Bcl-XL | MR-133a/-326 by directly targeting Bcl-XL could co-contribute to HCC cell 5-FU sensitivity. | (90) |
| miR-141 | – | HepG2, Huh7,SMMC-7721, HepG2/5-FU, SMMC-7721/5-FU, Huh7/5-FU | Keap1, Nrf2 | MR-141 by repressing Keap1 could confer 5-FU resistance and contribute to reduced susceptibility to 5-FU-induced apoptosis via activating Nrf2-dependent antioxidant pathway. | (91) |
| miR-145 | mouse/human: 102 pairs of HCC and ANTs | SNU449, Huh7, LO2 | TLR4 | MR-145 by targeting TLR4 could enhance chemosensitivity in HCC cells. | (92) |
| miR-147 | mouse/human: 10 pairs of HCC and ANTs | HepG2, C3A, SNU-398, Hep3B, THLE2, THLE3, Huh7, MHCC97L, MHCC97H | HOX6 | MR-147 by inhibiting HOX6 could suppress HCC cell proliferation, migration and enhance chemosensitivity to 5-FU. | (88) |
| miR-193a-3p | mouse | OGY-7703, SMMC-7721, BEL-7402, HepG2, Hep3B, PLC, YY-8103, FOCUS | SRSF2, E2F1 | DNA methylation-regulated miR-193a-3p by repressing SRSF2 could dictate resistance of HCC cells to 5-FU. | (93) |
| miR-195 | – | BEL-7402, BEL-7402/5-FU | Bcl-w | MR-195 by targeting Bcl-w could confer HCC cells to 5-FU-induced apoptosis. | (94) |
| miR-200a-3p | – | Hep3B | DUSP6 | MR-200a-3p by regulating DUSP6 expression could increase 5-FU resistance in Hep3B cells. | (95) |
| miR-302b | – | SMMC-7721, HepG2 | Mcl-1, DPDY | MR-302b by targeting Mcl-1 and DPDY could enhance the sensitivity of HCC cells to 5-FU. | (96) |
| miR-503 | 9 HCC and ANTs | HepG2, BEL-7402, SMMC-7721, L-02 | Eif4E | MR-503 by targeting Eif4E could render HCC cells susceptible to 5-FU and inhibit cell proliferation. | (97) |

FBXO31, resulting in suppression of FBXO31 and activation of p38 MAPK. Systemic administration of miR-29c has significantly enhanced response to 5-FU in xenograft models of esophageal cancer (109). In cervical cancer, miR-138/-135 can target FAK, enhance 5-FU sensitivity, and inhibit invasion and tumor growth (110). In pancreatic cancer cells, miR-221-3p, miR-486-5p, miR-21, and miR-320a influence response to 5-FU through targeting RB1, PTEN, and PDCD4 (111–114). Finally, in chronic myeloid leukemia (CML), miR-378 suppresses FUS1 expression, promotes cell proliferation, inhibits apoptosis, and confers resistance to 5-FU (115). Table 4 provides an overview of researches that studied the role of miRNAs in the modulation of response to 5-FU in other types of cancer.

The expression pattern of several miRNAs that influence response to 5-FU is associated with the survival of patients with malignancies. Among oncogenic miRNAs, over-expression of miR-1229-3p in gastric cancer patients has been associated with shorter overall and relapse-free survival rates (108). On the other hand, down-regulation of several tumor-suppressive miRNAs such as miR-488, miR-145, and miR-199b has been associated with poor survival of cancer patients (43, 77, 92). The possible application of 5-FU-associated miRNAs as diagnostic markers has also been assessed showing the diagnostic power values of 0.807 and 0.77 for miR-1229-3p and miR-378 in gastric cancer and CML, respectively (108, 115). Table 5 provides a summary of the importance of 5-FU-related miRNAs as diagnostic or prognostic markers in cancers.

**LNCRNAS AND RESPONSE TO 5-FU**

Expression of HOTAIRM1 has been decreased in CRC tissues and cell lines, particularly in 5-FU resistant tissues and cells. In 5-FU resistant CRC cells, this lncRNA has been shown to suppress cell progression through acting as a competing endogenous RNA for miR-17-5p, thus enhancing the expression of BTG3 (123). HOTAIR is another lncRNA that induces resistance to 5-FU in CRC. This lncRNA down-regulates mir-218 level via an EZH2-related mechanism. HOTAIR silencing has suppressed cell viability and arrested cells in the G1-phase through enhancing miR-218 expression. VOPP1 is a functional target of this miRNA. Moreover, NF-kB signaling has been shown to be inhibited by HOTAIR via repression of miR-218 expression. Inactivation of NF-kB signaling by HOTAIR silencing has also partly reversed 5-FU resistance. Another route of participation of HOTAIR in resistance to 5-FU is the enhancement of TS expression (124). A high throughput assessment of transcriptome in 5-FU-resistant CRC cells and parental cells has revealed differential expression of more than 2,000 lncRNAs which have been enriched in Jak-STAT, PI3K-Akt, and NF-kB signaling pathways, emphasizing the role of these pathways in conferring resistance to 5-FU (125). Table 6 summarizes the role of lncRNAs in the modulation of response to 5-FU in CRC. Figure 2 illustrates that 5-FU-induced changes in cell cycle regulation of several cancer cells might be associated with an alteration of G1 and G2 target genes expression through the modulation by various non-coding RNAs.
In addition to CRC, lncRNAs have fundamental roles in conferring resistance to 5-FU in other types of cancer, particularly gastric cancer and HCC. For instance, in gastric cancer cells, SNHG20 has been shown to mediate resistance to 5-FU via modulating the expression of miR-140-5p and subsequent up-regulation of its target gene NDRG3 (138). The role of PVT1 in the progression of this kind of cancer and induction of chemoresistance is mediated via up-regulation of the antiapoptotic gene Bcl2 (139). KRAL is a down-regulated lncRNA in HepG2/5-FU and SMMC-7721/5-FU cells compared with the corresponding parental cells. Up-regulation of KRAL has enhanced Keap1 expression. The resistance of these cells to 5-FU could be reversed through the inactivation of the Nrf2-dependent antioxidant pathway. KRAL serves as a sponge for miR-141 and subsequently restores Keap1 expression (140). NR2F1-AS1 is involved in the modulation of response to 5-FU in breast cancer cells. This lncRNA increases IGF-1 levels via sponging miRNA-338-3p and then activates IGF-1R and ERK pathways (141). TMPO-AS1 is an over-expressed lncRNA in ovarian cancer tissues and SKOV3 cells. This lncRNA regulates TMEFF2 via sponging miR-200c. Moreover, it activates the PI3K/Akt signaling pathway. TMPO-AS1 silencing has suppressed epithelial–mesenchymal transition (EMT), invasiveness, migration and 5-FU resistance in ovarian cancer cells (142). Table 7 summarizes the role of lncRNAs in modulation of response to 5-FU in other cancers.

In gastric cancer, Kaplan–Meier analysis has demonstrated that patients with over-expression of PVT1 do not benefit from 5-FU containing chemotherapeutic regimens. However, additional studies in CRC FIGURE 1 | A schematic representation of the crosstalk between microRNAs and the Wnt/β-catenin pathway contributing in the modulation of 5-FU in the cancer cell. Mounting evidence has indicated that microRNAs dysregulation and the Wnt/β-catenin signaling pathway jointly drive the regulation of the sensitivity of tumor cells to 5-FU as a chemotherapeutic agent. As an illustration, miR-30-5p has been detected to function as a tumor suppressor via regulating the Wnt/β-catenin signaling cascade in colorectal cancer cells. miR-30-5p could downregulate the expression level of Wnt/β-catenin signaling target genes (MYC and Axin2) and the levels of β-catenin protein, thereby promoting the sensitivity of these target cells to 5-FU agent (48). Besides, mir-125b is a critical downstream mediator of the CXCL12/CXCR4 axis which could activate the Wnt/β-catenin signaling via targeting the APC gene and could play an effective role in enhancing invasion and 5-FU resistance by elevating autophagy in colorectal cancer cells (101).
have shown the importance of expression levels of several lncRNAs namely HOTAIR, PCAT6, UCA1, XIST, TUG1, HAND2-AS1, LINC00152, and H19 in the determination of patients’ prognosis (Table 8). Figure 3 depicts the regulation of the efficacy of 5-FU-based chemotherapy in cancer cells via multiple non-coding RNAs through the Notch signaling cascade.

### APPLICATION OF THE CRISPR/CAS9 SYSTEM WITH THE AIM OF OVERCOMING 5-FU RESISTANCE IN HUMAN CANCER CELLS

Accumulating evidence revealed that the CRISPR-Cas9 gene-editing tool can be considered as a potential approach in order to promote sensitivity to chemotherapeutic agents. Due to the reason that gene mutation plays a remarkable role in developing drug resistance in tumor cells, CRISPR-Cas9 can be employed as an effective gene manipulation system with regards to permanently removing genes and attenuating resistance to cancer chemotherapy (149–151). Furthermore, this applicable method can be applied effectively and has a great advantage compared to other gene-editing technologies such as siRNAs, ZFNs, and TALENs in manipulating target genes involved in the chemotherapy resistance (152–154). Clinical evidence demonstrated that cartilage oligomeric matrix protein (COMP) has a substantial part in tumorigenesis, proliferation, and invasion of colon cancer cells. Utilizing the CRISPR/Cas9 system, it has been possible to create COMP-knockout cells via lentiviral vectors which could, in turn, enhance the sensitivity of tumor cells to 5-FU and suppress PI3K/Akt/mTOR/p70S6K pathway (155). In addition, Lobo et al. figured out that employing the CRISPR/Cas9 gene-editing tool for manipulation of CD44 in addition to Phosphorodiamidate Morpholino oligomers (PMOs) can promote cisplatin and 5-FU sensitivity in gastric tumor cells (156). Furthermore, due to the association of GPRC5a with a worse prognosis, the CRISPR/Cas9 was employed to knock-out the expression level of this target gene to reduce proliferation and migration ability of tumor cells and inhibit resistance to oxaliplatin, 5-FU, and gemcitabine in pancreatic cancer cells (157). Moreover, current research indicated that upregulation of MUC5AC could widely affect colorectal cancer chemotherapeutic response via overexpression of β-catenin and its target genes CD44 and Lgr5 as well as suppression of p53 and its target gene p21, which is frequently associated with aggressiveness of colorectal cancer cells. RNA interference and CRISPR/Cas9 systems were utilized to knock-out the expression of MUC5AC in tumor cells thereby enhancing the sensitivity of cancer cells to 5-FU and oxaliplatin (158). With the emergence of the CRISPR-Cas9, experimentations in the field of drug resistance in various human cancers have been advanced greatly. A summary of clinical researches related to the knock-out of various genes causing 5-FU resistance in several human cancer cells via the CRISPR/Cas9 gene-editing tool is demonstrated in Table 9. Although these studies have targeted mRNA coding genes, they show the feasibility of targeting certain transcripts and the significant effects of these methods in sensitization of neoplastic cells to 5-FU. Similar strategies targeting lncRNAs/miRNAs would have similar effects on cancer cells.
EFFECT OF HISTONE DEACETYLASE INHIBITORS IN COMBINATION WITH 5-FU ON PROMOTING THE CHEMOTHERAPEUTIC EFFICACY IN MULTIPLE HUMAN CANCERS

Accumulating evidence has demonstrated that tumorigenesis not only occurs by a genetic mutation, but it also could be triggered via epigenetic alteration processes. Modification of histones by acetylation has an important part in epigenetic modulation of gene expression which is regulated by both histone acetyltransferases (HAT) and histone deacetylases (HDAC). Since dysregulation of histone acetylation is a hallmark of cancer in some cases, thereby employing HDAC inhibitors could shed novel insights into regulatory mechanisms of transcription as well as inducing differentiation and apoptosis. HDAC inhibitors are potential anticancer drugs because of their ability to induce tumor cell differentiation, cell cycle arrest, and cell death, attenuate angiogenesis, reverse transformed cell morphology, and regulate immune response. Importantly, the combination of HDAC with 5-FU can elevate the efficacy of this agent in tumor cells. The HDAC inhibitor SAHA has a key role in promoting sensitivity to 5-FU and irinotecan via triggering proapoptotic signals in hepatocellular carcinoma cells through overcoming MDR-mediated drug efflux, suppressing SN-38 glucuronidation and synchronization of the cell cycle by upregulation of CDK-inhibitor p21cip1/waf1.

Additionally, Minegaki et al. have demonstrated that co-administration of 5-FU with VPA or SAHA as an inhibitor of histone deacetylases could downregulate the expression level of TS in 5-FU-resistant MDA–MB-468 breast cancer cells, and thereby promoting the sensitivity of both 5-FU-sensitive and resistant breast cancer cells to 5-FU chemotherapy. Moreover, another research has illustrated that the combination of HDAC inhibitor depsipeptide and 5-FU could significantly enhance the sensitivity of colon cancer cells to chemotherapy. This sensitization of tumor cells could occur via triggering cell cycle arrest caused by overexpression of p21 (CDKN1A), modulating apoptosis represented by caspase-3/7.
TABLE 5 | Diagnostic/prognostic roles of 5-FU-related miRNAs (OS, overall survival; RFS, relapse-free survival; DFS, disease-free survival).

| Sample | Area Under Curve | Sensitivity | Specificity | Kaplan-Meier | Univariate/Multivariate Cox regression analysis | Ref |
|--------|-----------------|-------------|-------------|--------------|-----------------------------------------------|-----|
| 60 GC patients | 0.807 | 73.7 | 80.5 | High level of miR-1229-3p was associated with shorter OS and RFS rates. | A high level of miR-1229-3p was correlated with advanced TNM stages. | (108) |
| 280 CRC patients | – | – | – | Low level of miR-488 was associated with shorter survival rate. | – | (77) |
| 102 HCC patients | – | – | – | A low level of miR-145 was associated with a shorter survival rate. | A low level of miR-145 was correlated with lymph node metastasis and advanced TNM staging. | (92) |
| 110 LARC patients | – | – | – | Low level of miR-199b was associated with shorter OS and RFS rates. | – | (43) |
| 97 CRC patients | – | – | – | Low level of miR-552 was associated with shorter OS and DFS rates. | – | (81) |
| 104 ESCC patients | – | – | – | Low level of miR-338-5p was associated with shorter survival rate. | – | (117) |
| TCGA dataset | – | – | – | Low level of miR-29c was associated with shorter OS rate. | – | (122) |
| 59 CML patients (miR-378) | 0.770 | 72.1 | 90.9 | – | – | (115) |
| 56 CRC patients | – | – | – | Low level of miR-329 was associated with shorter OS. | – | (72) |
| CRC patients from PROGenev2 database | – | – | – | Low level of miR-29c-3p was associated with shorter OS and DFS rates. | – | (47) |
| 30 melanoma patients | – | – | – | Low level of miR-204-5p was associated with shorter survival. | – | (119) |
| 152 CRC patients | – | – | – | A low level of miR-145 was associated with shorter survival. | – | (59) |

**Activation as well as regulating the expression level of MHC class II (175). In addition, Hamam et al. have detected that the effect of 5-FU against colon tumor cells could be promoted remarkably by the combination treatment with CUDC-907, a dual HDAC, and PI3K inhibitor. This could in turn lead to upregulation of apoptotic processes and necrosis, as well as enhancing polyploidy (176). Besides, Tan et al. have shown that the HDAC6 selective inhibitor ACY1215 could play an effective role in inhibiting colon cancer cell growth, migration, invasion, and triggering apoptosis in colon cancer cells, and thereby elevating the efficacy of 5-FU (177). On the other hand, another study showed that upregulation of HDAC4 could modulate TGFβ signaling cascade via reducing the expression levels of SMAD4, SMAD6, BMP6, Id1, TGFβ2, and TGFβ3 in breast cancer cells. HDAC4 could also regulate the expression of SMAD4 via histone h3 deacetylation which could in turn augment MDA-MB-231 cell resistance to 5-FU (178). The effects of epigenetic regulators on the expression of lncRNAs/miRNAs which are linked with cellular response to 5-FU have been less studied.**

**The Role of Autophagy in 5-FU Treatment in Multiple Human Cancers**

Multidrug resistance (MDR) could occur mostly after long-term chemotherapy, leading to tumor recurrence. Autophagy, a self-degradative mechanism, generally occurs during the process of resistance to chemotherapy. Autophagy can enhance the MDR and protection of tumor cells from these drugs. Autophagy induced by anticancer agents could also trigger upregulation of apoptotic signaling pathways in MDR cells, simplifying MDR reversal (179–181). Accumulating evidence illustrated that suppression of autophagy by either pharmacological procedures or through regulatory gene silencing enhances 5-FU–induced tumor cell death. Furthermore, autophagy could have a pro-death role which may modulate cell death in various tumor cells to trigger apoptosis pathways. Therefore, autophagy could be a target to improve the sensitivity of multiple cancer cells to 5-FU (20). Zhang et al. have illustrated that a combination of 5-FU and β-Elemene could play an effective role in promoting the sensitivity of p53-deficient colorectal cancer cells to 5-FU via modulation pro-death autophagy by promoting the formation of autophagosome (182). Furthermore, another research has demonstrated that psilostachyin-A can attenuate 5-FU resistance in liver carcinoma via triggering autophagy in these cells. Psilostachyin-A could cause the enhancement of the autophagosomes via upregulating the expression levels of LC3B-II and Beclin-1 in the HepG2 cells. This could also induce G2/M arrest of the tumor cells through declining of cyclin B1 and CDK1 expression as well as suppressing the MAPK/ERK signaling cascade, and thereby inhibiting proliferation and invasion of the HepG2 cells to the large extent (183). Besides, Zhang et al. have detected that whilst
TABLE 6 | Role of IncRNAs in the modulation of response to 5-FU in colorectal cancer (ANT, adjacent normal tissue).

| IncRNA     | Human/Animal               | Assessed Cell line | Targets/Regulators | Function                                                                 | Ref  |
|------------|----------------------------|--------------------|--------------------|---------------------------------------------------------------------------|------|
| HOTAIRM1   | athymic/ human: 56 pairs of CRC and ANTs | HCT116, SW480, NCM460, HCT116/5-FU, SW480/5-FU | miR-17-5p, MRP1, MDRI, BTG3, E-cadherin, N-cadherin | HOTAIRM1 via sponging endogenous miR-17-5p/BTG3 axis could suppress cell progression in 5-FU resistant CRC cells. | (123) |
| HOTAIR     | 48 pairs of CRC and ANTs | HT29, SW480, FHC, HT29/5-FU, HCT116, SW620, SW1116, lovo, RKO, color205 | miR-218, VOPP1, TS, AKT, ERK, E2F-1, NF-kB | HOTAIR via suppressing miR-218 and activating NF-κB/TS signaling could contribute to 5-FU resistance. | (124) |
| uc010vzg.1 | Microarray                 | HCT116, HCT116/5-FU | JAK/STAT, PI3K/AKT, NF-κB | Any change in IncRNA expression could be involved in 5-FU-based CRR in CRC cells. | (125) |
| PCAT6      | 73 pairs of CRC and ANTs | HCT116, HT-29, SW620, SW480, DLD-1, RKO, LoVo, 293T, CCD-112CoN | miR-204, HMG2A, PI3K/AKT | Overexpression of PCAT6 by inhibiting miR-204 thereby promoting HMG2A/Pi3K axis could enhance the chemoresistance of CRC cells to 5-FU. | (126) |
| NEAT1      | 55 pairs of CRC and ANTs | FHC, HT29, HCT8, HCT116, SW480, SW620 | miR-34a, Caspase-3, LC3 II/I, ULK1, Beclin-1, ATG9A, ATG4B, HMG2B | NEAT1 silencing could attenuate autophagy to elevate 5-FU sensitivity in CRC. | (127) |
| NEAT1      | male BALB/c-nude mice/human: 30 pairs of CRC and ANTs | SW480, HCT116, NCM460 | miR-150-5p, CPSF4, P-gp, GST-π | NEAT1 via the miR-150-5p/CPSF4 axis could regulate 5-FU sensitivity in CRC. | (128) |
| ENST00000547547 | – | HCT116, LoVo, LoVo/5-FU, HCT116/5-FU | miR-31, Bax, Bcl-2 | ENST00000547547 via competitive binding to miR-31 could reduce the 5-FU resistance of CRC cells. | (129) |
| UCA1       | 119 pairs of CRC and ANTs | 293T, HCT8, HCT116, HT29, LoVo, SW480, SW620 | miR-204-5p, CREB1, Bcl-2, RAB2A | UCA1 by inhibiting miR-204-5p could increase cell proliferation and 5-FU resistance in CRC. | (130) |
| XIST       | 268 pairs of CRC and ANTs | HT29, HCT116, HFC, HT29/5-FU, HCT116/5-FU | TS | XIST via promoting thymidylate synthase expression could inhibit 5-FU-induced CRC cell cytotoxicity. | (131) |
| TUG1       | 124 pairs of CRC and ANTs | HCT8fu, HCT8, HCT116, SW1116 | miR-197-3p, TYMS | TUG1 by acting as a ceRNA of miR-197-3p could mediate 5-FU resistance in CRC. | (132) |
| HAND2-AS1  | nude mice/human: 27 pairs of CRC and ANTs | NCM460, HCT116, SW480, HCT116/5-FU, SW480/5-FU | miR-20a, PDCD4, Bax, Bcl-2, MMP2, MMP9 | HAND2-AS1 by modulating miR-20a/PDCD4 axis could inhibit 5-FU resistance in CRC. | (133) |
| LINCO0152  | nude BALB/c mice/human: 108 pairs of CRC and ANTs | HCT8, HT29, LoVo, HCT116, SW480, SW620, 293T | miR-139-5p, NOTCH1 | LINCO0152 by inhibiting miR-139-5p could promote cell proliferation and confer 5-FU resistance in CRC. | (134) |
| H19        | 110 pairs of CRC and ANTs | HCT8, HCT8fu, SW1116, 293T, HCT116, lovo, HT29, SW480, SW620, CCD-112CoN | Caspase-3, PAPAP, P62, LC3II, SIRT1 | H19 by promoting SIRT1-mediated autophagy could confer 5-FU resistance in CRC. | (135) |
| CCAT1      | BALB/c mice/human: 67 pairs of CC and ANTs | HCT116, SW1417, HT-29, KM12, NCM460 | γ-H2AX, p53, c-Myc | Downregulation of CCAT1 could enhance 5-FU sensitivity in CC cells. | (136) |
| H19, UCA1  | – | HCT116, DLD1, SW480, HCT116/5-FU, DLD-1/5-FU, SW480/5-FU, HCT116/p, DLD-1/p, SW480/p | Rb, p27kip1 | Overexpression of UCA1 and H19 could be involved in the impaired cell cycle in cells susceptible to 5-FU. | (137) |

Autophagy could be activated by treatment of human colon carcinoma cells with 5-FU, treatment of these cells with curcumin followed by the 5-FU treatment could considerably attenuate autophagy activation and promote the cytotoxicity of this chemotherapeutic drug. This could occur via the alteration in LC3II/LC3I, beclin-1, and p62 expression levels in cancer cells which could, in turn, contribute to the downregulation of Akt/mTOR as well as AMPK/ULK1 signaling cascades in HCT116 cells (184). Furthermore, Yu et al. have represented that miR-125b could enhance 5-FU resistance in colorectal cancer cells via promoting cell autophagy. miR-125b remarkably upregulates the expression levels of beclin-1 and cleaved LC3B-II which could, in turn, play an important role in triggering autophagy and reducing the sensitivity of cancer cells to 5-FU. MiR-125b was increased by the activation of the CXCL12/CXCR4 axis, and thereby miR-125b could significantly augment EMT. Inhibition of this miRNA may be a suitable approach to attenuate the development of chemoresistance in tumor cells which could play a critical role in the regulation of autophagy (101). Similarly, several miRNAs/IncRNAs that affect response to 5-FU modulate autophagy in the neoplastic cells.

DISCUSSION

A vast body of literature has revealed the impact of non-coding RNAs in the determination of the response of cancer cells to 5-FU. CRC and HCC are the most assessed cancer types in this regard possibly because of the vast application of this chemotherapeutic
agent in these types of cancer. Yet, the influence of non-coding RNAs in the modulation of response to 5-FU has been mostly assessed in vitro needing confirmation in animal models and human subjects. Autophagy has been identified as the main route of the function of non-coding RNAs in the determination of the response of cancer cells to 5-FU. Moreover, the influence of non-coding RNAs on apoptotic-related pathways also affects the response of cancer cells to 5-FU. EMT is also influenced by these non-coding RNAs. The latter function of lncRNAs and miRNAs is consistent with the observed association between EMT and acquired resistance to 5-FU in cancer cells (185).

It is crucial to emphasize that the results of in vitro studies regarding the role of non-coding RNAs in the modulation of response to 5-FU should be verified in animal models as well as...
human subjects. Although the results of these three types of studies are mostly consistent, there are few examples of inconsistency. For instance, while miR-23a antisense has enhanced the activation of the caspases-3 and -7 and increased the 5-FU-associated apoptosis in CRC cells, this approach has not improved the anticancer impact of 5-FU in the xenograft model of CRC (42). The field has shown the significance of systemic administration of miR-200c-5p/NDRG3 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells. Knockdown of TMPO-AS1 via the miR-200c/TMEFF2 axis could promote chemoresistance of PDAC cells. Overexpression of DGC5 via targeting miR-320a/PDCD4 axis could promote 5-FU resistance of PDAC cells.

Exosome-mediated transfer of non-coding RNAs particularly miRNAs is implicated in conferring chemoresistance at a wide distance from the original cells. Moreover, these cell-free particles can modulate several cells in the tumor microenvironment in favor of tumor progression. On the other hand, it is possible to take advantage of exosomes as vehicles for the specific transfer of anti-cancer agents to cancer cells. A successful example of the latter function of exosomes has been provided by simultaneous delivery of 5-FU and miR-21 inhibitor oligonucleotide to Her2 expressing cancer cells via engineered exosomes (186).

LncRNAs mostly affect the response of cancer cells to 5-FU through the modulation of the expression of miRNAs. HOTAIRM1/miR-17-5p, HOTAIR/miR-218, PCAT6/miR-204, NEAT1/miR-34a, NEAT1/miR-150-5p, ENST00000547547/miR-31, UCA1/miR-204-5p, TUG1/miR-197-3p, HAND2-AS1/miR-20a, KRAL/miR-141, SNHG20/miR-140-5p/NDRG3 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells. Knockdown of TMPO-AS1 via the miR-200c/TMEFF2 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells. Knockdown of TMPO-AS1 via the miR-200c/TMEFF2 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells. Knockdown of TMPO-AS1 via the miR-200c/TMEFF2 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells. Knockdown of TMPO-AS1 via the miR-200c/TMEFF2 axis and disrupting the PI3K/Akt signaling could inhibit the invasion, metastasis, and drug resistance of OC cells.
TABLE 8 | Prognostic roles of 5-FU-related lncRNAs (ANTm adjacent normal tissue; OS, overall survival; RFS, relapse-free survival).

| Sample | Kaplan–Meier | Multivariate Cox regression analysis | Ref |
|--------|--------------|-------------------------------------|-----|
| 48 pairs of CRC and ANTs | Higher expression of HOTAIR was related to lower OS and RFS rates. | Higher expression of HOTAIR was related to tumor size, distant metastasis, and tumor differentiation. | (124) |
| 73 pairs of CRC and ANTs | Higher expression of PCAT6 was related to a lower OS rate. | Higher expression of HOTAIR was related to TNM stage, tumor differentiation, and lymph node metastasis. | (125) |
| 119 pairs of CRC and ANTs | Higher expression of UCA1 was related to a lower OS rate. | Higher expression of UCA1 was related to tumor size and lymph node invasion. | (130) |
| 268 pairs of CRC and ANTs | Higher expression of XIST was related to lower OS and RFS rates. | Higher expression of XIST was related to TNM stage and distant metastasis. | (131) |
| 124 pairs of CRC and ANTs | Higher expression of TUG1 was related to lower OS and RFS rate. | Higher expression of TUG1 was related to the depth of the tumor. | (132) |
| 27 pairs of CRC and ANTs | Lower expression of HAND2-AS1 was related to lower OS rate. | – | (133) |
| 108 pairs of CRC and ANTs | Higher expression of LINC00152 was related to lower OS and DFS rates. | Higher expression of LINC00152 was related to the tumor stage. | (134) |
| 110 pairs of CRC and ANTs | Higher expression of H19 was related to lower OS and RFS rate. | – | (135) |
| 168 pairs of GC and ANTs | Higher expression of HOTAIR was related to lower OS rate. | Higher expression of HOTAIR was related to tumor size and TNM stage. | (143) |

FIGURE 3 | A schematic illustration of the Notch signaling pathway involved in the regulation of response of cancer cells to 5-FU via various non-coding RNAs. Notch signaling cascade is involved in the various processes of normal morphogenesis, such as cell growth, apoptosis, as well as the acquisition of drug resistance. LINC00152 could elevate tumor cell migration and invasion, and confer 5-FU resistance in colorectal cancer via modulating the expression level of NOTCH1 through sponging miR-139-5p and downregulating its function from enhancing CRC development (134). Additionally, miR-34a acts as a tumor suppressor and could directly downregulate the expression level of DLL1 as a ligand of the Notch signaling cascade, and thereby could inhibit tumor growth under 5-FU treatment by promoting chemosensitivity to this agent (51).
| Cancer Target | Cell line | Animal | In vitro | In vivo | CRISPR Vector | Other gene-editing methods | Treatment Signaling Effect | Ref |
|---------------|-----------|--------|----------|---------|---------------|-----------------------------|--------------------------|-----|
| Colorectal cancer (CRC) | MUC5AC | + | HCT-8, LS174T | 5-6-week-old athymic nude mice | Knockout targeting exon 2 | Lentiviral siRNA | 5-fluorouracil (5-FU), Oxaliplatin 5-FU | (159) |
| CRC SNHG15 | + | LoVo | 6-7-week-old female BALB/c- Rag2/-IL2cre/ immunodeficient mice | Knockout deleting the region between exon 3 and 5 | plasmid siRNA | CD44/b-catenin/ p53/p21 | Sensitized the cells | (159) |
| CRC CYSLTR1 | + | HT-29, HT-29-R | 4-week-old male BALB/c nude mice | Knockout targeting exon 10 | Lentiviral | LTD/ CysLT-R PI3K/Akt/ mTOR/ p70S6K JAK/Stat, ERK/ MAP, AMPK PTEN, PI3K/AKT | Sensitized the cells | (160) |
| CRC COMP | + | HEK 293T, LoVo, SW1116 | 6-week-old female BALB/c nude mice | Knockout | Lentiviral | 5-FU | Sensitized the cells | (155) |
| CRC BAG3 | + | HCT-116 | 6-week-old female BALB/c nude mice | Knockout targeting exon 10 | Lentiviral | 5-FU | Sensitized the cells | (161) |
| CRC LINC01021 | + | HCT116 | 6-week-old female BALB/c nude mice | Knockout targeting exon 10 | Plasmid siRNA | 5-FU, Doxorubicin | Sensitized the cells | (162) |
| CRC FoxO3A | + | HCT116 | 6-week-old female BALB/c nude mice | Knockout targeting exon 2 | Plasmid siRNA | 5-FU, Irinotecan, Cisplatin, Etoposide | Sensitized the cells | (163) |
| Gastric cancer (GC) cd44v6 | + | MKN45, GP202 | 6-week-old female BALB/c nude mice | Editing targeting Exon-v6 Splice-Sites | Plasmid PMOs | 5-FU, Cisplatin | Sensitized the cells | (164) |
| GC GSDME | + | MKN-45, SGC-7901 | 6-week-old female BALB/c nude mice | Knockout targeting exon 2 | Plasmid siRNA | 5-FU, MEK/ ERK, AMPK | Sensitized the cells | (165) |
| Metaplasia DDIT4 | + | MGC-803 | 6-week-old female BALB/c nude mice | Knockout targeting exon 2 | Plasmid | 5-FU, mTORC1 | Sensitized the cells | (166) |
| Myeloid malignancies (MDS, AML) ASXL1 | + | U937 | 6-week-old female BALB/c nude mice | Frameshift mutation targeting a specific site (nt1010-1031) of exon 8 | Plasmid | 5-FU, Cisplatin | Sensitized the cells | (167) |
| Nasopharyngeal carcinoma (NPC) EBV DNA | + | C666-1, HEK293M81 | 6-week-old female nude (Eso26 and OE19), NOD-SCID IL-2RyKO mice (FLO-1) | Knockout targeting exon 6 | Lentiviral siRNA | 5-FU, Cisplatin, Epirubicin | Sensitized the cells | (168) |
| Oesophageal adenocarcinoma (OAC) TP53 | + | OE33, OE19, H1299, HEK293T, JH-EsoAd1, FLO-1, OACM5.1, Eso26, SKGT4, OACP4C, TE7, OANC1, NES | 6-week-old female nude (Eso26 and OE19), NOD-SCID IL-2RyKO mice (FLO-1) | Knockout targeting exon 6 | Lentiviral siRNA | 5-FU, Cisplatin, Epirubicin, P53 | Sensitized the cells | (169) |
merely reflect the oncogenic or tumor-suppressive effects of these transcripts.

**Perspectives and Future Directions**

Generally, this review provides convincing evidence for the role of miRNAs and IncRNAs as biomarkers of response to 5-FU treatment in a variety of solid tumors, especially in colorectal cancer cells. Patients with gastrointestinal cancer become resistant to 5-FU because of the aberrant expression of certain genes. This event is a prevalent phenomenon in clinical practice. Modulation of expression levels of miRNAs or IncRNAs may be a suitable approach to sensitize tumor cells to 5-FU treatment via modulating multiple biological signaling pathways like Hippo/YAP, Wnt/β-catenin, Hedgehog, NF-kB, and Notch cascades. There is an increasing interest in targeting miRNAs as well as IncRNAs in various kinds of cancers that are treated by 5-FU. However, due to the wide range of miRNAs and IncRNAs regulating the response to 5-FU and their aberrant expression in multiple cancers, it is required to characterize the most clinically relevant non-coding RNAs in these malignancies. Therefore, researchers should systematically investigate the correlations between genes, pathways, and drug sensitivity to find direct causal effects. Besides, the research procedures recently utilized are mainly phenotype-based, like *in vitro* cell proliferation, migration and invasion, and *in vivo* mouse specimens. To find practical strategies, novel gene editing systems such as the CRISPR/Cas9 method should be applied to figure out the biological role of various target genes as well as non-coding RNAs in human cancers. Additionally, the enhancement of human primary cell models and patient-derived tumor xenograft (PDX) animal models may also play a key role in scrutinizing the role of non-coding RNAs and improving non-coding RNA-targeting techniques. We also suggest that non-invasive liquid biopsies such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) should be employed to identify factors that are explicitly accompanied with 5-FU sensitivity and/or adverse reactions. These methods can help in the reduction of ineffective therapies and overdose as well as attenuating toxic side effects of 5-FU. Moreover, based on sufficient experimental data, we propose that the procedure of downregulating autophagy by either pharmacological methods or via silencing genes involved in the autophagy could also be considered as effective adjunctive therapy to improve the sensitivity of tumor cells to 5-FU. Besides, we propose that epigenetic processes such as modification of histones by acetylation can influence response to 5-FU. The obtained information from these studies will guide the advancement of precision medicine in the upcoming future.

**AUTHOR CONTRIBUTIONS**

MT and SG-F supervised the study, wrote the draft, and edited the submission. HS, AA, FT, FF, and SJ performed the data collection and designed the tables and figures. All of the authors contributed equally and fully aware of submission. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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