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REVIEW

# An Update on the Potential Roles of E2F Family Members in Colorectal Cancer



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**Abstract:** Colorectal cancer (CRC) is a major health burden worldwide, and thus, optimised diagnosis and treatments are imperative. E2F transcription factors (E2Fs) are a family of transcription factors consisting of eight genes, contributing to the oncogenesis and development of CRC. Importantly, E2Fs control not only the cell cycle but also apoptosis, senescence, DNA damage response, and drug resistance by interacting with multiple signaling pathways. However, the specific functions and intricate machinery of these eight E2Fs in human CRC remain unclear in many respects. Evidence on E2Fs and CRC has been scattered on the related regulatory genes, microRNAs (miRNAs), and competing endogenous RNAs (ceRNAs). Accordingly, some drugs targeting E2Fs have been transferred from preclinical to clinical application. Herein, we have systemically reviewed the current literature on the roles of various E2Fs in CRC with the purpose of providing possible clinical implications for patient diagnosis and prognosis and future treatment strategy design, thereby furthering the understanding of the E2Fs.

**Keywords:** E2Fs, colorectal cancer, proliferation, apoptosis, drug resistance

## Introduction

Cancer is a major leading cause of death in the 21st century globally. Despite remarkable advancements that have been made in the diagnosis and treatment of CRC in recent years, the number of new cases of colorectal cancer (CRC) reached 1.8 million across the world in 2018, with 881,000 reported deaths.[1] Furthermore, about 30–50% of patients exhibit local recurrence or metastasis after radical resection.[2] Since, the principal obstacles to CRC treatment are tumor recurrence, metastasis, and resistance, the 5-year survival rate remains less than 65%.[3,4] Unfortunately, the classic biomarkers have limited predictive and clinical value. Thus, there is an urgent need to discover novel diagnostic and prognostic biomarkers for this lethal disease.

In 1986, it was found that the E2F transcription factors (E2Fs) could bind to the promoter of the adenoviral gene E2. Based on their molecular structure and transcriptional properties, the E2F family can be categorized into three groups: transcriptional activators (E2F1, E2F2, and E2F3a), canonical repressors (E2F3b and E2F4-E2F6), and atypical repressors (E2F7 and E2F8).[5] The E2Fs are becoming increasingly complex owing to several E2F isoforms, including two splice variants of E2F3a (E2F3c and E2F3d).[6] The E2Fs had come to the frontiers of cancer research when they were found to be regulated by the retinoblastoma gene product, composed of pRB (RB1), p107 (RBL1), and p130 (RBL2).[7,8] Aberrant

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E2F transcriptions have been identified in many human malignancies. Mechanistically, dysregulated E2Fs can activate or silence some oncogenes or tumor suppressors at multiple levels, including transcriptional level, post-transcriptional level, translational level, protein-protein interaction level, and transcriptional activity level, and further causing the carcinogenesis in human malignancies, including CRC.[9] Importantly, in addition to the classic cell-cycle-intrinsic regulation, E2Fs control apoptosis,[10] senescence,[11] DNA-damage response,[6,12] autophagy,[13] metabolism,[9] angiogenesis,[9] and drug resistance.[14] (see Figure 1) However, the specific functions and intricate machinery of these eight E2Fs in human CRC remain unclear in many respects. Thus, further studies need to be comprehensively reviewed for a greater understanding of their detailed regulatory mechanisms in CRC. In this review, we have systematically searched Web of Science, EMBASE, PubMed, Wanfang, China National Knowledge Infrastructure (CNKI), VIP databases, and SinoMed databases to investigate the current state of knowledge of the roles of various E2Fs in CRC with the purpose of providing possible clinical implications for patient diagnosis, prognosis, and future treatment strategy design.

## E2F-Related RNAs in CRC

Long non-coding RNAs (lncRNAs) are RNA transcripts with a length of more than 200 nucleotides (nts).[15] LncRNAs regulate microRNAs (miRNA) as competing endogenous RNAs (ceRNAs). For instance, colorectal neoplasia differentially expressed (CRNDE) promotes metastasis and oxaliplatin resistance by hijacking miR-136 and regulating E2F1 expression in CRC.[16] Similarly, SNHG6, located at chromosome 8q13.1, acts as a ceRNA by sponging miR-181a-5p, promoting E2F5-mediated proliferation of CRC cells.[17] Recently, the E2F1-mediated MNX1-AS1-miR-218-5p-SEC61A1 feedback network was discovered to be also pivotal for CRC tumorigenesis.[18] Oncogenic H19, another interesting lncRNA, is an independent predictor of CRC survival. It interacts with macroH2A and promotes CRC growth and migration by targeting RB1/E2F1 signaling and cyclin-dependent kinases (CDK)-β-catenin activity.[19]

Several circular RNAs (circRNAs) also exhibit oncogenic properties by functioning as ceRNAs. For example, MAT2B, a novel circRNA, was found to increase E2F1 expression through sponging miR-610, resulting in tumorigenesis or further development.[20] Similarly, cir_001569 upregulates E2F5 by sponging miR-145 and is correlated with the aggressive character of CRC.[21] Furthermore, the circCAMSAP1/miR-328-5p/E2F1 axis is also essential for CRC progression.[22]

MiRNAs are key components of the multi-level regulatory system. They are a class of short (20–22 nts) non-protein-coding endogenous RNAs that regulate CRC oncogenesis by binding to complementary sequences (3′-untranslated regions, 3′-UTRs) of target mRNAs to direct their post-transcriptional repression.[23–27] For example, both miR-342-3p and miR-377 target the E2F1 3′-UTRs to inhibit the proliferation of glioma cells.[28] Additionally, miR-526b-3p is related to a better prognosis in CRC patients and directly targets the 3′-UTRs of E2F1 mRNA, leading to reduced E2F1 expression.[29] It is noteworthy to mention that unique miRNAs expression profiles can be observed in different stages of the CRC progression.[30] Similarly, miR-4711-5p dramatically induces G1 arrest by downregulating the downstream molecules of the E2F-TFDP1 complex in HCT-116 cells, including cell division cycle protein 6 (CDC6), CDT1, and MCM7.[24] MiR-106a and miR-362-3p are two other promising miRNAs that act as negative upstream regulators of E2F1 and improve patient survival.[31,32] It has been well documented that miR-31 and miR-155 drive CRC development by decreasing E2F2.[33,34] This suggests that they might become targets for anti-tumour drug design. Furthermore, some known miRNAs, such as miR-194,[26] miR-377,[35] miR-449b,[36] and miR-503,[37] play a role in growth suppression through modulating E2F3 in CRC. It has been reported that miR-34a might serve as the key upstream negative regulators of E2F1, E2F3, and E2F5 and enhanced 5-Fluorouracil (5-FU) cytotoxicity in CRC.[38–42] The 1,2-diaminocyclohexane carrier ligand-mediated p53-miR-34a-E2F signaling pathway also appears to have an important mechanism.[42] Moreover, circulating miR-34a combined with miR-150 has been reported to be capable of distinguishing patients with polyps, adenomas, and advanced cancer.[43] The miR-3666 has been identified as a tumor suppressor in breast cancer[44] and thyroid carcinoma.[45] Specifically, the miR-3666/E2F7 is suggested to play a crucial role in modulating HCT116 cell viability, apoptosis, and migration by inhibiting the signaling activation of mitogen-activated protein kinase (MAPK)/extracellular regulated protein kinases (ERK).[46] MiR-1258 negatively controls E2F8 by influencing several cell-cycle factors, including cyclin D1 and cyclin-dependent kinase inhibitor 1A in CRC.[47]

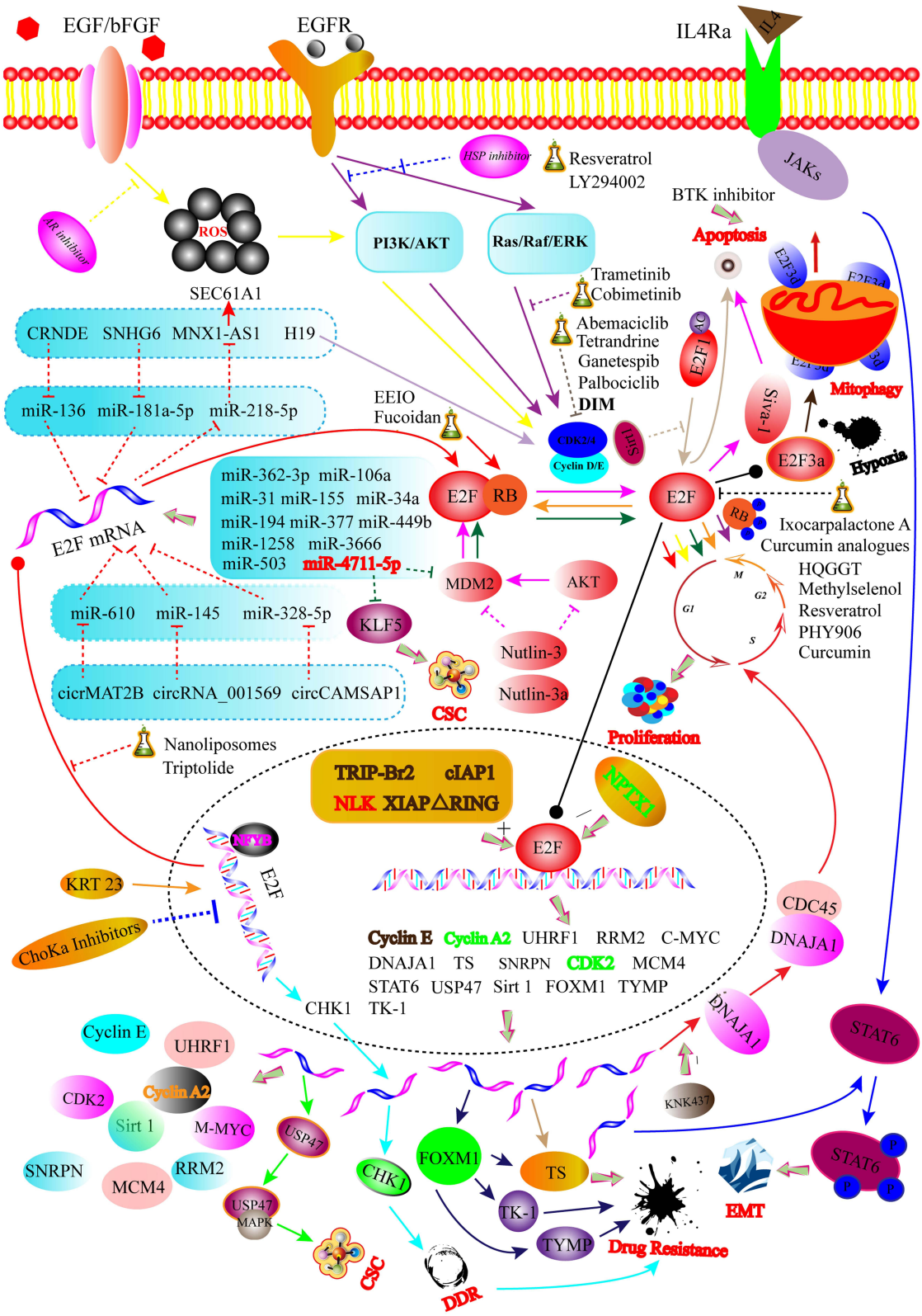


**Figure 1** Graphical illustration showing that the regulatory mechanisms for E2Fs in CRC. For instance, E2Fs function in CRC is modulated via multiple levels including the transcriptional level (NFYB, KRT23, and ChoKa inhibitors-mediated transcription of the E2F gene), post-transcriptional regulation (E2F mRNA targeted by different miRNAs and ceRNAs), post-translational modifications (deacetylation and acetylation of E2F protein), protein–protein interaction level (phosphorylation and dephosphorylation of RB protein), and transcriptional activity level (XIAPΔRING, TRIP-Br2, cIAP1, NLK, and NPTX1 regulate the transcriptional activity of E2Fs protein). Solid arrows represent promoted effects, while dashed arrows represent inhibitory effects. Different colored lines showed different signaling pathways or targets.

**Abbreviations:** DDR, DNA damage response; CSC, cancer stem cell; EMT, epithelial-mesenchymal transition.

However, the miRNAs targeting E2F4 and E2F6 in CRC have not yet been discovered.

In cancer cells, the E2F-miRNA regulatory loops have been described.[27,48] For instance, miR-26a and E2F7 constitute a reciprocal regulatory network in which miR-26a inhibits E2F7 expression, while E2F7 targets MYC and decreases miR-26a.[48] Similarly, E2F7 suppresses miR-199b expression in SW403 cells, and miR-199b targets ubiquitin-specific protease 47 (USP47) that stabilizes MAPK, promoting colon cancer stem cell activity and subsequently accelerating colon cancer progression.[49] Furthermore, Gao et al summarized the bidirectional cross-link between E2F3 and 29 miRNAs in human cancers and elucidated how this regulation occurs.[27] This review unfolds a series of RNA interaction profiles (see Figure 1). Collectively, we think the miRNA-based cancer therapeutic method is a promising next-generation treatment strategy since miRNAs can be readily detected in various biofluids and tissues, such as blood, serum, plasma, saliva, and stools.[50,51]

## Upstream or Downstream Proteins Involved in E2Fs Regulation in CRC

It has been well known that E2Fs can either activate or inhibit gene transcription, depending on the cell type, the target genes, the expression levels of co-regulator partners, and the external environment.[52–54] In particular, the CDK-RB-E2F pathway is important for cell fate determination. More specifically, the CDK and cyclin complexes phosphorylate RB and release E2Fs. Re-establishing cell cycle regulation through direct or indirect inhibition of CDK is suggested as an attractive option of the molecularly targeted therapy.[55,56] (see Figure 1) As reported, the CDK4/6 inhibitors in human CRC are currently being tested, including tetrandrine,[56] abemaciclib,[57] ganetespib,[58] and palbociclib.[59] These inhibitors inhibit tumorigenesis, at least partially by reducing the expression of E2F target genes. (see Figure 1) Former studies focused on regulatory components modulating the CDK-RB-E2F axis and gained lots of valuable insights.[9] Particularly, over-expressed TRIP-Br2 was found to promote anchorage-independent growth of HCT-116 cells by activating the RB/E2F/DP1-mediated transcription through upregulation of cyclin E, cyclin A2, CDC6, and DHFR of the key E2F-responsive partners.[60] Likewise, the cellular inhibitor of apoptosis 1 also seems to be crucial for optimal E2F1 mediated-cyclin A and cyclin E expression.[54] Another powerful gene, X-linked inhibitor of apoptosis protein (XIAP) with RING (Really Interesting New Gene) domain deletion ($XIAP^{\Delta RING}$) translocates into nuclear and promotes cancer cell-autonomous growth by targeting the E2F1/cycle E axis.[61] Moreover, Neuronal pentraxin 1 (NPTX1), a member of the long pentraxin family (NPTX1, NPTX2, and NPTXR), suppresses the growth of colon cancer cells through decreasing cyclin A2 and CDK2 expression.[62] As reported, histone deacetylases (HDACs) function as the negative regulator of E2F1 through deacetylation. Nemo-like kinase boosts CRC progression by releasing E2F1 from the E2F1/HDAC1 complex.[63]

Importantly, the cross-talk between the RB/E2F and Wnt/β-catenin signaling pathways in human malignancies has already been characterized.[64] Identification of the critical effectors of the cross signaling pathway is beneficial for CRC management. In particular, E2F1 suppresses Wnt/β-catenin activity through inhibitor of β-catenin and TCF4 (ICAT). Phospholipase D1 (PLD1) controls the cross-link among E2F-miR-4496 and Wnt/β-catenin pathways and the tumor-initiating program of CRC cells.[65] Furthermore, PLD1 also regulates the Wnt/β-catenin signaling by selectively downregulating ICAT via the Phosphoinositide 3-Kinase (PI3K)/Akt-TopBP1-E2F1 signaling axis.[66,67]

E2Fs are targeted by different proteins (see Figure 1). For instance, Keratin 23 is strongly expressed in colon adenocarcinomas compared to normal colon mucosa, and its depletion leads to a reduced expression of many key molecules including E2F1.[68] Spinophilin is a previously recognized novel tumor suppressor gene. Ress et al proposed that spinophilin expression modulates cellular growth, cancer stemness, and 5-FU resistance in CRC cells by inhibiting E2F1 activation.[69] ChoKα specific inhibitors, MN58b and TCD-717 modulate the expression levels of TS and TK1 through the inhibition of E2F production.[70] Aldose reductase (AR), an NADPH-dependent Aldo ketoreductase, is involved in colon carcinogenesis. Ramana et al reported that inhibition of AR inhibits the related growth factor-induced G1-S phase transition via the AKT/PI3K/E2F1 signaling pathway in human colon cancer cells.[71,72]

E2Fs activates numerous downstream regulatory genes (see Figure 1). E2F1 overexpression has been identified to promote the transformation of aggressive phenotypes in CRC cells by activating the ribonucleotide reductase small subunit M2.[73] Ubiquitin-like with PHD and ring-finger domain 1 (UHRF1) expression has been discovered to be

upregulated by E2F1 and involved in the cellular proliferation of CRC. Particularly, enhanced UHRF1 expression appears to be involved in carcinogenesis of the right compared to the left hemicolon.[74] The MDM2 antagonists nutlin-3 and nutlin-3a can induce cancer cell apoptosis in a p53-dependent manner.[42,75,76] Interestingly, they also initiate apoptosis by activating E2F1- and p73-mediated expression of Siva-1 and p53 upregulated modulator of apoptosis (PUMA) regardless of p53 status in CRC.[75] Moreover, CDCA3 is referred to as a trigger of mitotic entry, mediates p21-dependent proliferation of CRC by regulating E2F1 expression.[77] It has been reported that KNK437 is a heat shock protein inhibitor that inhibits the DNAJA1-induced CRC proliferation and metastasis.[78] Mechanistically, DNAJA1 is activated by E2F1 and then promotes the cell cycle by stabilizing CDC45. More importantly, the combined application of KNK437 with 5-FU/L-OHP shows a synergistic inhibitive effect on DNAJA1-mediated liver metastasis. Li et al found that E2F2 acts as a tumor suppressor in CRC by repressing CCNA2, C-MYC, CDK2, and MCM4.[33] A recent study also demonstrated that the small nuclear ribonucleoprotein polypeptide N accelerates the malignant progression of CRC regulated by E2F8.[79]

## Immune Microenvironment and E2Fs in CRC

The tumor microenvironment (TME) constitutes immune cells, stromal cells, and extracellular matrix, which functions as an immunologic battleground for tumor cells and the immune system during tumor formation.[80] The TME and the related inflammatory response play an imperative role in cancer development and progression. It should be noted that chronic intestinal inflammation such as inflammatory bowel disease promotes pRB hyperphosphorylation and E2F1 activation, directly increasing the CRC risk.[81–84] Multiple pro-inflammatory cytokines, including interleukin (IL)-6, IL-8, IL-13, and IL-17 released by diverse infiltrating cells, such as neutrophils, macrophages, and lymphocytes, have been found to induce CRC metastasis.[82,85–88] Remarkably, Chen et al proposed a model that the E2F1/SP3/STAT6 axis induced by IL-4 promotes the epithelial-mesenchymal transition (EMT) of CRC cells.[89] (see Figure 1) The microbiota has been identified as an important part of TME. It has been reported that changes in the microbiota in TME mediate chronic inflammation and CRC initiation.[90] Thompson et al found H. influenza to be significantly related to genes in the G2M checkpoint, E2F transcription, and mitotic pathways in breast cancer.[91] Similarly, commensal gut microbiota also shapes the colonic immune environment in CRC.[92] However, little is known about the relationships between E2Fs and microbiota in CRC.

The human genome is constantly exposed to both endogenous and exogenous stresses, such as hypoxia, ionizing radiation, and acidosis, which can lead to genomic instability and the subsequent increased mutation rate, thereby accelerating the tumorigenesis.[93–99] Changes in the TME, such as hypoxia and nutrient deprivation has been discovered to cause mitochondrial damage.[100] Araki et al found that a distinctive product, E2F3d triggers the hypoxia-induced fragmentation and mitophagy in cancer cells.[6] Furthermore, hypoxia causes elevated mutagenesis,[94] diminished capacity of DNA repair,[94] reduction in the expression of the key mismatch repair genes, MLH1[95] and MSH2,[97] and of the homologous recombination (HR) gene.[99] Mechanistically, E2Fs could mediate the down-regulation of BRCA1 or RAD51 expression in response to hypoxic stress and consequently suppress HR activity.[98,99] Moreover, overexpression of RAD51 is considered to be a poor prognostic predictor in CRC patients.[101]

Cancer stem cells (CSCs) are a small subset of cells in tumors with the potential of self-renewable, differentiation, and tumor-initiation.[102,103] Emerging evidence suggests that adult stem cell populations with high proliferation rates have a higher cancer rate than less proliferative stem cell populations independently of oncogene expression.[104,105] Importantly, around two-thirds of mutations in cancer are caused by replicative errors. E2F family members sometimes work in opposition, including copy number gains of E2F1 and E2F3 or copy number deletion of E2F7 and E2F8, inducing cancer in mice.[106,107] One promising miRNA, miR-4711-5p, can inhibit CSC properties by downregulating Kruppel-like factor 5 expression and MDM2.[24] Similarly, the E2F7-regulated miR-199b/USP47/MAPK axis promotes the stemness of colon CSCs[49] (see Figure 1).

## E2F-Induced Metabolic Dysregulation in CRC

Metabolic reprogramming is considered an emerging hallmark of cancer. Accordingly, several metabolism-targeted therapies have been proven to the promising anti-tumor

strategies. It is known that the cellular metabolic changes may precede somatic mutations in CRC. For example, oncogene activation and tumor suppressor loss further reprogram CRC cells and upregulate glycolysis, glutaminolysis, one-carbon metabolism, and fatty acid synthesis.[108] Mutated metabolic features occur in CRC at multiple levels, including tumor cells, CSCs, TME, and host–microbiome interactions.[108] E2Fs have been reported to contribute to global metabolic homeostasis in a cell-cycle independent manner. E2F1 promotes glycolysis, lipogenesis, bile acid synthesis, and insulin secretion in related normal cells.[109] Especially, the mentioned TRIP-Br2 and CDK4-pRB-E2F1 are vital for adipogenesis and maintaining adipocyte function.[109,110] Conversely, E2F1 has been reported to repress lipolysis, thermogenesis, and oxidative metabolism of cancerous cells and contribute to the Warburg effect.[109,111,112] It is important to note that insulin receptor substrate-4 is overexpressed in CRC cells and increases the RB-cyclin-dependent kinase pathway.[113] Although E2Fs is rarely reported in the metabolic signaling pathway of CRC, the above-mentioned studies suggest a possible three-way interaction between E2Fs, metabolism, and CRC.

## E2Fs-Target Drugs in CRC

Some pharmacological agents at least partially modulate the CRC progression by targeting E2Fs (see Table 1 and Figure 1). The siE2F1 loaded cationic nanoliposomes (small unilamellar vesicles, SUVs) have been found to exhibit very low cytotoxicity in human CRC cell lines and be effective in silencing E2F1 and in the consequent reduction of cell growth.[114] Developing plant-derived products as potential anticancer agents has attracted considerable interest in recent years throughout the world. For instance, resveratrol,[115,116] brassinin,[117] eguelin,[118] tetrandrine,[56] ethanol extract of *Inonotus obliquus*,[119] and the non-digestive fraction of beans[120] have been identified as anti-tumor agents. Especially, 3,3′-Diindolylmethane (DIM), as one of the natural indole derivatives originating from broccoli and other cruciferous vegetables, has been shown to exert antitumor effects in both in vivo and in vitro models. Choi et al indicated that DIM restricted CDK2 activity and RB phosphorylation, reducing the levels of the E2F1 protein in HT-29 human colon cells.[121] Furthermore, ixocarpalactone A isolated from the Mexican tomatillo was found to manifest potent antiproliferative and apoptotic activity in SW480 cells by modulating E2F1 and Bcl-2 family.[122] Recently, a herbal formulation Huang Qin Ge Gen Tang (HQGGT), was discovered to enhance 5-FU cytotoxicity and antitumor activity through the suppression of the E2F1/TS signaling pathway in CRC.[123] Generally, curcumin induced reactive oxygen species down-regulation of E2F4 expression and consequently lead to apoptotic cell death in HCT116 colon cancer cells.[124] In addition, the curcumin and its analogues EF31 and UBS109 induce apoptosis and inhibit growth by downregulating E2F1 and its target gene thymidylate synthase (TS).[125] Similarly, cobimetinib, a MEK inhibitor seems to improve the efficacy of 5-FU by decreasing TS.[126] Fucoidan, a natural sulfated polysaccharide that exists in brown seaweed, exerts anticancer effects by inhibiting pRB phosphorylation and enhancing binding pRB with E2Fs in HCT116 cells.[127] In general that triptolide can initiate programmed cell death by activating apoptosis or autophagy.[128] More interestingly, its water-soluble analogue named minnelide induces cell death by apoptosis at low concentrations and E2F-dependent G1 phase arrest at higher concentrations.[129] Likewise, traditional Chinese medicine PHY906,[130] methylselenol,[131] and irinotecan[132] serve oncogenic roles by decreasing the expression of E2Fs.

## Resistance and E2Fs in CRC

Currently, surgery and chemoradiotherapy (CRT) are considered to be standard treatment options for CRC. However, the fact that most patients develop resistance to standard therapies poses a significant challenge in the treatment of CRC.[133] Therefore, it is crucial to elucidate the underlying mechanisms for CRT in clinical practice.

Mounting evidence suggests that enhanced E2F activity is a key mechanism of the CRT resistance (see Figure 1). For instance, E2F1 regulates multiple downstream target genes that are related to DNA synthesis and repairs genes that are involved in resistance, including the BRCA1, RAD51, TS, excision repair genes (ERCC-1), and forkhead box M1 (FOXM1).[134–136] It is no exaggeration that 5-FU is the backbone of CRC first-line therapy and exerts crucial anti-tumor activities, at least partially, via E2F1/TS downregulation.[134,136–139] This signaling pathway can be interrupted by diverse stimuli, including glycogen synthase kinase 3β (GSK-3β) inhibitor (2′Z,3′E)-6-bromo- indirubin-3′ -oxime (BIO),[140] curcumin analogues (EF31 and UBS109),[125] as well as RB-reactivating agents (trametinib (MEK inhibitor), fenofibrate (PPARα agonist), and LY294002 (PI3K inhibitor)).[141] Of note, the combined E2F1+TS+immunophenotype in CRC manifests a poor prognosis.[139] These findings suggest that the

**Table 1** E2Fs-Target Agents Summary in CRC

| Agents | Properties | Target | Anticancer Effects | Reference |
|---|---|---|---|---|
| Nanoliposomes | SUVs loaded with siE2F1 | E2F1 | Inhibiting the growth of colon carcinoma cells. | [116] |
| Resveratrol | Polyphenolic compound (isolated from grapes, peanuts or berries) | E2F1<br>E2F3 | 1. Enhancing the growth inhibition of colon carcinoma cells and cell apoptosis by targeting miR-34a/E2F3/Sirt1 and PI3K/Akt signaling pathway. 2. Enhancing the TTP inhibitory activity in CRC cells by negatively regulating cIAP2, E2F1, LATS2 and Lin28 expression. | [117,118] |
| Tetrandrine | Bis-benzylisoquinoline alkaloid (isolated from the root of Stephania tetrandra) | E2F1 | Inducing early G1 arrest by downregulating E2F1 and upregulating p53/$p21^{Cip1.}$ | [58] |
| Ethanol extract of Innotus obliquus | Bioactive compounds | E2F1 | Inducing G1 cell arrest and inhibiting cell proliferation by decreasing RB phosphorylation and E2F1 expression. | [121] |
| Non-digestible fraction of beans | Common beans extraction | E2F1 | Exhibiting apoptosis induction, cell-cycle arrest, inhibition of cell proliferation and inflammation and induction of DNA repair. | [122] |
| 3,3′-Diindolylmethane | Indole derivative | E2F1 | Inducing G1 and G2/M phase cell cycle arrest mediated by reduced CDK activity and E2F1. | [123] |
| Ixocarpalactone A | A withanolides extract from Mexican tomatillo | E2F1 | Exerting potent antiproliferative and apoptotic activity in SW480 cells by modulating E2F1 and Bcl-2 family. | [124] |
| HQGGT | Traditional Chinese Herbal Medicine | E2F1 | Suppressing CRC cell growth and promoting apoptosis in vivo and vitro xenografts; enhanced CRC cell sensitivity to 5-FU via suppressing the E2F1/TS signaling pathway. | [125] |
| Curcumin | A polyphenolic phytochemical isolated from the plant Curcuma longa | E2F4 | ROS produced by curcumin is responsible for the cell growth inhibition and the downregulation of E2F4 expression. | [126] |
| EF31 and UBS109 | Curcumin analogues | E2F1 | Inducing cell cycle arrest through downregulation of signaling proteins | [127] |
| Cobimetinib | MEK inhibitor | E2F1 | Inhibiting cell proliferation and inducing G1 phase arrest and apoptosis in HCT116 cells; enhancing the efficacy of 5-FU. | [128] |
| Fucoidan | Natural sulfated polysaccharide present in various brown algae | E2Fs | Exhibiting anticancer effects through the induction of cell cycle arrest and apoptosis regardless of the p53 status. | [129] |
| Triptolide/ Minnelide | A natural compound isolated from the Chinese herb Tripterygium wilfordii/Its soluble analog | E2F1 | Inducing apoptosis at low concentrations and E2F-dependent G1 phase arrest at higher concentrations. | [130,131] |
| PHY906 | Traditional Chinese Herbal Medicine | E2F1 | Protecting the epithelial barrier against tumor cell invasion by modulating IFN-γ level and mediating cancer cell death. | [132] |
| Methylselenol | A selenium metabolite | E2F1 | Regulating the expression of key genes related to cell cycle and apoptosis and inhibiting colon cancer cell proliferation and tumor growth. | [133] |
| Irinotecan | Topoisomerase I inhibitor | E2F1 | Overcoming the resistance to 5-FU in combination with 5-FU pro-drugs on 5-FU-resistant colon tumors. | [134] |

**Abbreviations:** SiE2F1, siRNA against the transcription factor E2F1; SUVs, small unilamellar vesicles; TTP, tristetraprolin; cIAP2, baculoviral IAP repeat containing 3; Lin 28, lin-28 homolog A; LATS2, large tumor suppressor kinase 2; HQGGT, Huang Qin Ge Gen Tang; TS, thymidylate synthase; ROS, reactive oxygen species; MEK, mitogen-activated protein kinase.

downregulation of TS expression might be a promising method of improving the efficacy of 5-FU. Recently, Lavitrano et al identified a novel oncogenic isoform of Bruton's tyrosine kinase, namely p65BTK.[142] Silencing p65BTK was found to overcome the 5-FU resistance of CRC cell lines and restore the E2F-dependent apoptosis. The aberrant activation of nuclear transcription factor Y subunit beta (NFYB)-E2F1-checkpoint kinase 1 (CHK1) was identified to maintain the tumorigenicity in oxaliplatin-resistant CRC and significantly related to a poor prognosis.[143] Chen et al revealed that antagonism of CDK8 inhibits the fractional survival of CRC cells and increases radiotherapy-induced apoptosis in vivo and in vitro through potentiating the transcription of E2F1 target gene apaf1.[144] Apart from their roles in CRC carcinogenesis, MiRNAs may also be involved in affecting the chemosensitivity by targeting E2Fs in CRC. For example, miR-329 attenuates the chemoresistance of CRC to 5-FU by degrading E2F1.[145] Similarly, miR-34a enhances the sensitivity of human CRC cells to 5-FU by inhibiting Sirt1 and E2F3, which is correlated with inactive PI3K/AKT signaling pathway.[39,40] The liposomal miR-34a mimic, MRX34, is the first synthetic miRNA that has been already entered into clinical trials, providing a proof-of-concept for mi-RNA-based cancer therapy.[51] Some miRNAs, such as miR-200b, miR-21, and miR-192, were successively found to induce apoptosis and restore chemosensitivity in an E2F-dependent manner. Recently, Lin et al have constructed an adenoviral vector (AdCMVE2F-1) to transfected an ectopic E2F1 into human CRC cells. The findings showed that the upregulated E2F1 exerts a synergistic anticancer effect with gemcitabine.[146] It was suspected that the apoptotic effect of E2F1 is due to its unscheduled entry into the S phase. Importantly, the exogenous E2F1 exhibits clinical chemosensitizing effects in CRC cells by inducing pro-apoptotic behavior.[147,148] In conclusion, targeting the inhibition of E2F or killing oncogenes that drive E2F activity could be a good complement to current treatment strategies.[9]

## Conclusions and Perspectives

Taken together, the E2Fs are a quite complex family of the transcription factor. They have been found to appear in many emerging fields of CRC in addition to classic cell-cycle regulation, such as CSCs, TME, and metabolism. Importantly, they can exert different biological functions depending on context. Some pharmacological agents indirectly or directly regulate the CRC progression by targeting E2Fs. Accumulating evidence has shown that enhanced E2F activity is a critical mechanism of CRT resistance in CRC. Therefore, targeting the inhibition of E2F or killing oncogenes that drive E2F activity could be a good complement to current treatment strategies.

However, further studies are warranted to more thoroughly examine the effect and mechanisms further of E2Fs in CRC.

1. The endogenous carcinogenic and exogenous pro-apoptotic effects of E2F1 are an ongoing paradox for the scientific community. How to explain the pro-apoptotic molecular mechanism of E2Fs in CRC?
2. How E2Fs specifically mediate metabolism and TME in CRC?
3. More clinical agents can be designed for targeting E2Fs, and the related CRT mechanisms can be further studied.

Collectively, E2Fs are thought to be a promising target in CRC. More prospective research is needed to verify this conclusion.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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