Review Article
A comprehensive review of the roles of E2F1 in colon cancer

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Abstract: E2F transcription factor 1 (E2F1) is a member of the E2F family of transcription factors. E2F1 binds to DNA with dimerization partner (DP) proteins through an E2 recognition site. The dissociation of E2F1 from retinoblastoma (Rb) protein recovers its transcriptional activity, which drives the cell cycle from the G1 to S phase. E2F1 has been shown to be involved in cellular proliferation, differentiation, and apoptosis in colon cancer. It was recently found that E2F1 also participates in the metastasis and chemoresistance of colon cancer. There are abundant experimental data regarding the actions of E2F1, which can be grouped as either pro-tumorigenic or pro-apoptotic. Despite a growing interest and plentiful data, there is currently no review that focuses on the role of E2F1 in colon cancer. Research on E2F1 and colon cancer has been scattered over various genes and microRNAs (miRNAs) that affect E2F1 expression. Here, we provide the first review that aims to consider and dissect all of the elucidated complex behaviors of E2F1 in colon cancer. This review also provides an analysis and conclusion regarding the current understanding of E2F1 in colon cancer in order to facilitate the direction of future research.

Keywords: E2F1, colon cancer, proliferation, chemoresistance, apoptosis

Introduction

An enhancer box (E-box) is a DNA element that responds to basic helix-loop-helix (bHLH) transcription factors and regulates the transcription of various genes [1]. E-protein dimers bind to E-box sequences (CANNTG) with an apparent affinity for C or G in N positions. The activity of E proteins is antagonized by inhibitors of DNA-binding (Id) proteins, which transform these proteins into nonfunctional heterodimers [2]. E2F transcription factors (E2Fs), first found to bind to the promoter of adenoviruses [3], are a group of transcription factors that play a pivotal role in cellular proliferation, differentiation, and apoptosis (**Figure 1**). Currently, eight E2F members have been named in order of their discovery from E2F1-E2F8 [4]. Intricate machinery of cell-cycle components-such as cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors, and *RB1*-provides impetus to downstream E2Fs, which results in their activation to enable cellular division and proliferation [4].

Among these eight factors, E2F1 was the first member discovered [5, 6]. E2F1 is a transcription activator that binds to DNA with dimerization partner (DP) proteins via the E2 recognition site, 5'-TTTC[CG]CGC-3', found in the promoter region of various genes. It is well established that E2F1 is a downstream target of the pocket protein, retinoblastoma (Rb) [7, 8], which is a master regulator of the cell cycle and a major tumor suppressor that is mutated in many human cancers [9]. A family of pocket proteins-including Rb protein (pRB), p109, and p130-has a binding specificity for E2F subunits, among which pRB preferentially binds to E2F1. The interaction of E2F1 with pRB leads to the suppression of the former in two different ways: (1) through binding and obstructing the transcriptional activation domain of E2F1; and (2) via
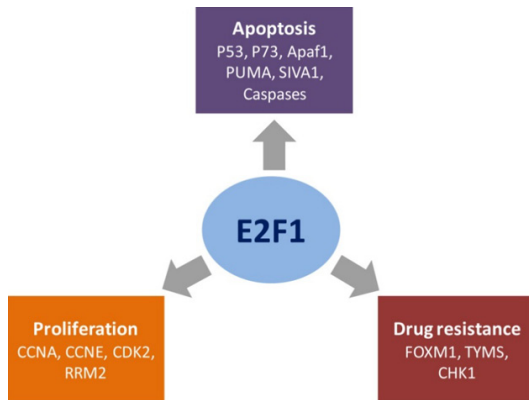
Figure 1. Different actions of E2F1 and their corresponding target genes in CRC. E2F1 has been reported to play crucial roles in proliferation, chemoresistance, and apoptosis of CRC through activating different downstream effectors.

recruiting histone deacetylases (HDACs) and SWI/SNF complexes, which change the structure of E2F-targeted genes [10-12]. Phosphorylation of Rb is indispensable for the release of active E2F1, which is required to drive the cell cycle from the G1 to S phase [13, 14]. Therefore, regulation of E2F1 is closely related to DNA replication and repair [15, 16]. After dissociation from pRB, E2F1 interacts with DP1 or DP2 to induce dNTP synthesis (by thymidylate synthase [TYMS] and ribonucleotide reductase M2), DNA synthesis (by DNA polymerase-α), damage repair (by ribonucleotide reductase M2b), and cell-cycle progression (by cyclin A, cyclin E, and cdk2) [17, 18]. Interestingly, E2F1 is directly implicated with poor prognosis in several types of cancer and has been demonstrated to be a key cancer biomarker [19, 20]. In breast cancer, overall survival and disease-free survival rates were significantly lower in E2F1-positive patients as compared to those in E2F1-negative patients [21]. Notably, in our previous work, increased E2F1 was required for the proliferation and metastasis of colorectal cancer (CRC) cells by inducing RRM2 expression, which also indicated poor survival in CRC patients [22]. Recently, we also found that high expression of E2F1 promoted the epithelial-mesenchymal transition (EMT) of CRC cells through augmenting IL4/STAT6 signaling [23]. Although E2F1 functions and their relationships with cancer have been investigated and discussed for years, we suggest that all previous studies need to be systematically reviewed and summarized to provide a better understanding of the many roles of E2F1 in CRC.

E2F1 in colon cancer

5-Fluorouracil and E2F1 in colon cancer

It is known that 5-fluorouracil (5-FU) has been widely used as a drug of choice for treating CRC [24, 25]. Specifically, 5-FU typically targets TYMS, which catalyzes the reaction from 2'-deoxyuridine monophosphate to deoxythymidine-5'-monophosphate (dTMP) using 5'10-methylene tetrahydrofolate, a precursor for DNA synthesis [26]. The expressions of TYMS and FOXM1 are closely controlled by E2F1 [27-29]. In a recent study, Varghese *et al.* demonstrated that the expression of FOXM1, TYMS, and E2F1 were elevated in CRC cells and promoted 5-FU resistance [26]. Likewise, Osada *et al.* evaluated the anti-cancer properties of 5-FU in hepatocyte growth factor (HGF)-stimulated CT26 CRC cells and found that treatment with HGF increased the 5-FU-induced death signal and inhibition of cellular growth. Mechanistically, it has been suggested that HGF decreases E2F1 by reducing cyclin D or E [30]. In addition, tissue microarrays from 190 CRC patients manifested a poor prognosis for the E2F1 + thymidylate synthetase (TS) + phenotype. More aggressive or different treatments than 5-FU-based chemotherapy are suggested in such subgroups of CRC patients [31]. Furthermore, Nagaraju *et al.* showed more sensitivity to 5-FU in CRC after transcriptional and functional inhibition of heat shock protein 90 (HSP90). Interestingly, inhibition of HSP90 leads to the downregulation of E2F1, which may confer the demonstrated response to 5-FU [32]. Taken together, these studies suggest that the anti-tumor activities of 5-FU in CRC work at least partly by decreasing E2F1 expression, which subsequently arrests the cell cycle. Although there are many reports that indirectly associate the effect of 5-FU with E2F1 expression, direct evidence is lacking.

E2F1-incited metabolic deregulation in colon cancer

Metabolic features of E2F1 have been described in both normal cellular metabolic machinery and metabolic reprogramming in cancerous cells. In normal cells, E2F1 was found to increase the synthesis of adipogenesis, glycolysis, lipogenesis, and bile acids. On the contrary, E2F1 was highlighted to reduce lipolysis, β-oxidation, thermogenesis, and oxida-
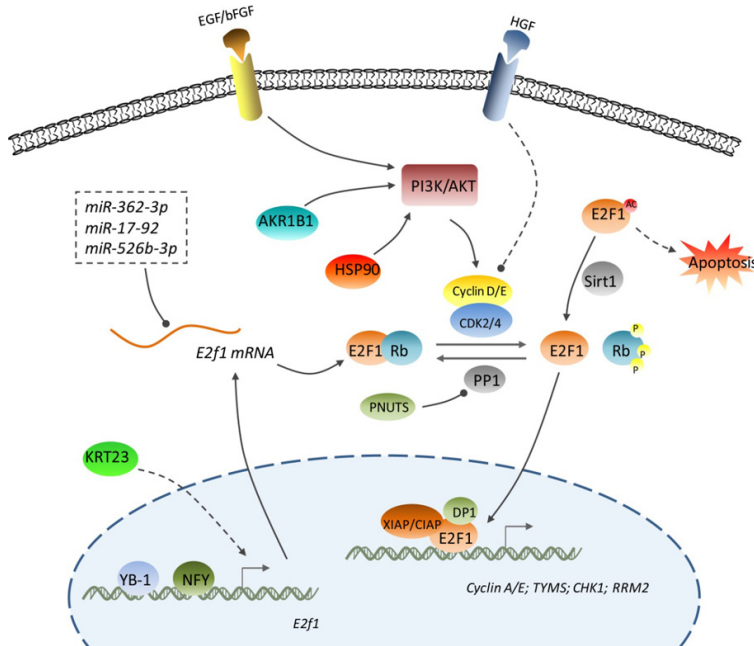
Figure 2. The regulatory mechanisms for E2F1 in CRC. E2F1 function in CRC is regulated at multiple levels including the transcriptional level (YB-1, NFY, and KRT23-induced transcription of the E2F1 gene), post-transcriptional level (E2F1 mRNA targeted by miR-362-3p, miR-17-92, and miR-526b-3p), post-translational level (deacetylation and acetylation of E2F1 protein exhibit opposite actions), protein-protein interaction level (dephosphorylated Rb sequesters E2F1 and hyper-phosphorylated Rb releases E2F1), and transcriptional activity level (XIAP, CIAP, and DP1 enhance the transcriptional activity of E2F1 protein). Arrowhead: promotion; Cycle: inhibition; Dashed line: indirect regulation.

tive metabolism [33]. Interestingly, all of these metabolic features affected by E2F1 are independent factors for general carcinogenesis [34] and, specifically, CRC [35]. More systematically, E2F1 has been reported to contribute to Warburg effects [36], repress oxidative metabolism [37], and promote anabolic metabolism [38, 39]. Consistent with these studies, Sanmartín-Salinas *et al.* recently found that insulin receptor substrate-4 (IRS-4) was overexpressed in CRC and promoted Rb-cyclin-dependent kinase activation *via* definitive involvement of E2F1 [40]. Although there is a lack of reported data on the metabolic pathways involving E2F1 in CRC, the above findings underscore a possible association among E2F1, metabolism, and CRC.

Proteins involved in E2F1 regulation in colon cancer

Many studies have highlighted different genes that contribute to the pro-tumorigenic effect of E2F1 in CRC (**Figure 2**). For instance, the tumor

suppressor, spinophilin, participates in tumor progression and indicates a poor prognosis in many different kinds of cancers [41, 42]. Ress *et al.* found increased cellular growth rates and anchorage-independent growth in p53 wild-type HCT116 and p53-mutated Caco-2 cells when spinophilin levels were low. Intriguingly, researchers discovered a parallel increase in E2F1 levels when spinophilin expression was low [43]. Another powerful gene, X-linked inhibitor of apoptosis (XIAP), has a well-established role in the modulation of cellular apoptosis. In 2014, Cao *et al.* showed that expression of XIAP with the Really Interesting New Gene (RING) domain omission (XIAPΔRING) stimulated the anchorage-independent growth and G1/S phase transition of cancer cells, in which XIAPΔRING increased binding with E2F1 in order to regulate its own transcriptional activity [44]. Another related gene, cellular inhibitor of apoptosis 1 (cIAP1), is localized in the nucleus and promotes the growth of various cancers [45, 46]. The presence of cIAP1 was observed in the nucleus of undifferentiated proliferating cells, but not in differentiated cells [47, 48]. A French team validated that the N-terminal part of cIAP1 interacts directly with the DNA-binding domain of E2F1 to increase the transcriptional activity of E2F1 on cyclin E (CCNE) and cyclin A (CCNA) promoters [49]. As a nucleic-acid-binding protein, Y-box-binding protein-1 (YB-1) has been demonstrated to be responsible for cancer development [50]. Knockdown of YB-1 with siRNAs significantly reduced the promoter activity of the E2F1 gene in the CRC cell line, HCT116 [51]. In our previous study, nuclear accumulation of nuclear transcription factor Y subunit β (NFYB) was found to directly activate the transcription of E2F1 in oxaliplatin-resistant CRC cells [52].

Keratin is a filament-forming epithelial protein with involvement in the proliferation, invasion, metastasis, and treatment responsiveness of

cancer cells [53, 54]. Keratin 23 (KRT23) is a type-1 acidic keratin exhibiting tumorigenic action in hepatocellular cancer [55] and colon adenocarcinomas [56, 57]. Birkenkamp-Demtröder et al. used array-based methylation profiling to reveal that normal colon mucosa had a methylated promoter of KRT23, as compared to the hypomethylated promoter in colon adenocarcinomas. Cellular proliferation and DNA damage responses were significantly curbed after KRT23 depletion in CRC cells; it was also revealed in the same report that KRT23 depletion led to a reduced expression of E2F1 [58], shedding light on a direct relationship of KRT23 and E2F1 in CRC. Moreover, the polyol pathway enzyme, aldose reductase (AR; AKR1B1), was shown to be crucial for reactive oxygen species (ROS) signals induced by growth factors (basic fibroblast growth factor [bFGF] and platelet-derived growth factor [PDGF]) and cytokines (TNF-α) [59]. Inhibition of AR prevented the growth of CRC cells, which resulted from suppressing G1/S phase proteins, such as E2F1, cdks, and cyclins [60]. Inverse relationships of E2F1 with other proteins have also been reported. Pregnane X receptor (PXR) attenuates the proliferation and tumorigenicity of CRC cells by inhibiting E2F1 expression [61]. Latini et al. reported that ABI3, a tumor suppressor, suppressed proliferation and induced senescence in cells, which was accompanied by a reduction in E2F1 levels in CRC cells [62]. A previous report demonstrated that exogenous E2F1 induction resulted in an increase in the activity of the bcl-2 promoter. Bcl-2 overexpression in PhIP-induced colon tumors was implicated along with involvement of beta-catenin, c-Myc, and E2F1 [63]. There is also evidence showing that chronic colon inflammation alone, in the absence of tumors, has higher levels of E2F1 along with hyperphosphorylated pRb, which poses a threat for cancer proliferation [64]. In addition, inflammatory bowel disease presents a potential link between inflammation and cancer due to the abnormal regulation of the cell cycle at the G1/S checkpoint. Russo et al. demonstrated increased expression of E2F1 and CycD1 in Crohn's disease explants stimulated by lipopolysaccharides (LPS) [65]. Relatedly, Lee and colleagues analyzed 320 human tumor samples and found a link between TS and E2F1 with gastroenteropancreatic (GEP) and neuroendocrine tumors (NETs) [66].

Furthermore, growth factors-including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor II (IGFs), platelet derived growth factor (PDGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF)-are usually overexpressed in the epithelial tissues of CRC [60, 67]. Thus, an increasing number of studies highlight the tumor-promotive effect of growth factors by upregulating vital cell-cycle proteins, such as cyclin D1, cyclin E, cdks, PCNA, and transcription factors, including E2F1 and c-myc, through activating PI3K/AKT signals [68, 69].

E2F1-associated miRNAs in colon cancer

A microRNA (miRNA) is a small non-coding RNA that functions as a post-transcriptional regulator. Previous work has described the relationship between miRNAs and E2F1 expression, which subsequently plays a pivotal role in CRC (**Figure 2**). This section reviews reports regarding miRNA types and their abilities to regulate E2F1. For instance, a Danish research group showed a positive correlation of miR-362-3p with improved prognosis in CRC patients and repressed expression of E2F1 when overexpressing miR-362-3p in CRC cells [70]. In addition, miR-17-92 and its target, E2F1, were found to have a similar expression pattern in human colon development and CRC, with both regulating cellular proliferation [71]. Recently, we found that yin and yang 1 (YY1)-mediated silencing of miR-526b-3p promoted CRC proliferation via increasing E2F1 expression [72]. An interesting observation regarding miRNAs and E2F1 in CRC patients was provided by Kanaan et al. in 2012. In this study, six miRNAs (miR-122, miR-181a, miR-146b-5p, let-7e, miR-17, and miR-143) were upregulated from non-neoplastic tissue to dysplasia, while being downregulated from dysplasia to cancer. The authors also found another six differentially expressed miRNAs affecting the TP53 pathway (miR-122, miR-214, miR-372, miR-15b, let-7e, and miR-17) as targets of E2F1 [73]. It is plausible that these miRNAs affect the E2F1-TP53 axis in order to exert their actions in CRC. In addition to miRNAs, the long non-coding RNA, MNX1-AS1, was upregulated by E2F1 and enhanced the malignancy of colon adenocarcinomas, in which E2F1 was bound to the promoter of MNX1-AS1 [74].

Pharmacological evidence for a role of E2F1 in colon cancer

Some pharmacological molecules that relieve colon cancer by influencing E2F1 expression provide an insightful perspective regarding the implication of E2F1 in colon cancer (**Table 1**). For example, tristetraprolin (TTP) is an AU-rich element-binding protein that destabilizes E2F1 mRNA [75]. It has been acknowledged that human cancers usually have lower levels of TTP. Lee *et al.* revealed that resveratrol inhibited the proliferation and invasion/metastasis of CRC cells via TTP-induced downregulation of E2F1 [76]. In addition, Gong *et al.* studied 3495 differentially expressed genes (DEGs) in cobimetinib-treated and untreated HCT116 cells and found that E2F1 was downregulated after treatment with cobimetinib, a drug that was found to increase the efficacy of 5-FU by decreasing TYMS expression in the same study [77]. In order to improve the therapeutic effect and counter 5-FU resistance in CRC, Liu *et al.* tested the herbal formula, Huang Qin Ge Gen Tang (HQGGT), in 5-FU-resistant cells (H630R1) and mouse colon cancer cells (MC38); HQGGT and 5-FU were found to exert synergistic effects through the modulation of the E2F1/TS pathway [78]. After studying another Chinese medicine, PHY906, Su *et al.* demonstrated E2F1 as a potential drug target in CRC by using the Gene Expression Omnibus (GEO) database [79]. In our recent work, a high level of E2F1 was detected in oxaliplatin-resistant CRC cells, which induced CHK1 expression, and knockdown of E2F1 undermined this resistance [52]. Furthermore, Bochicchio *et al.* developed nanoliposomes loaded with an siRNA against E2F1. These nanoliposomes were delivered into human colorectal adenocarcinoma cell lines and human intestinal biopsies. The vesicles loaded with siE2F1 were effective in reducing the growth of CRC cells [80]. Furthermore, triptolide is a natural compound isolated from *Tripterygium wilfordi,* which has a potent anti-tumor property against many types of cancers [81-83]. Oliveira *et al.* found that triptolide attenuated the xenograft growth and liver metastasis of CRC by suppressing the transcriptional activation of E2F1 [84]. Similarly, an ethanol extract of *Innotus obliquus* [85], methylselenol [86], irinotecan [87], a non-digestible fraction of beans (*Phaseolus vulgaris* L.) [88], SU9516 [89], ixocarpalactone [90], alpha-tocopherol succinate (TS) [91], and tetrandrine [92] yielded identical results, in which their anti-tumor properties in CRC were matched with the downregulation of E2F1.

E2F1 paradox in colon cancer

Aside from the pro-tumorigenic effects of E2F1, some studies have reported contradictory results that are worth further examination and provide an interesting counterpoint to the roles of E2F1 in colon cancer (**Figure 1**). In one such study, lentiviral-vector-mediated knockdown of CDK-8 led to a decreased metastasis and invasion of CRC cells with a parallel increase in E2F1 expression [93]. Lin *et al.* transfected an ectopic E2F1 adenoviral vector (AdCMVE2F-1) into human CRC cells, and E2F1 overexpression displayed a synergistic antitumor effect with gemcitabine [94]. It was suspected that an unwarranted entry into the S phase was responsible for the apoptotic action of E2F1. Similar results were supported by a previous study, highlighting a correlation between increased E2F1 expression and enhanced apoptosis of both SW620 and HT-29 CRC cells in vitro [95]. Earlier studies also demonstrated increased chemosensitization after E2F1 transfusion [96]. p53 is a tumor suppressor protein that plays a vital role in preventing cancers primarily through cell-cycle arrest and apoptosis [97]. It is also well known that mdm2 inversely regulates p53 expression by competing with its binding site, thereby disrupting its transcriptional activity [98]. In 2008, an American research group pointed out that Nutlin-3, an anticancer drug candidate, induced cellular apoptosis in a human CRC cell line by inhibiting pRb. Furthermore, they showed that the pro-apoptotic effect of Nutlin-3 was dependent upon p53 and E2F1 transcriptional activities partially through the inhibition of pRb [99]. Likewise, knockdown of phosphatase nuclear targeting subunit (PNUTS) in CRC cells promoted the efficacy of roscovitine by stimulating phosphatase activity toward pRb, which was dependent on the activity of E2F1 [100]. Previously, the same research group found that knockdown of PNUTS activated Rb phosphatase, subsequently causing Rb de-phosphorylation and E2F1-dependent apoptosis via caspase-8. This report also reported that the apoptotic mechanism did not rely on p53 [101]. A similar pro-apoptotic behavior of E2F1 via

Table 1. Summary of E2F1-targeted drugs in CRC

Agents	Properties	Effects on E2F1	Anti-tumor properties	Ref.
Resveratrol	Polyphenolic compound	TTP-induced destabilization of E2F1 mRNA	Inhibiting the proliferation and invasion/metastasis of CRC cells	(76)
Cobimetinib	MEK inhibitor	Downregulation of E2F1 mRNA	Decreasing cell viability and inducing cell cycle arrest and apoptosis in CRC cells; increasing the efficacy of 5-FU	(77)
HQGGT	Traditional Chinese herbal medicine	Downregulation of E2F1 mRNA and protein	Suppressing the *in vivo* and *in vitro* growth of CRC cells with induction of apoptosis; enhancing the cytotoxicity of 5-FU	(78)
Nanoliposomes (siRNA against E2F1)	siE2F1 loaded nanoliposomes with about 40 nm size	Downregulation of E2F1 mRNA and protein	Suppressing the growth of CRC cells	(80)
Triptolide	diterpene triepoxide from *Tripterygium wilfordi*	Inhibition of transcriptional activity of E2F1	Decreasing the *in vitro* growth of CRC cells; decreasing xenograft growth and liver metastasis of CRC	(84)
Ethanol extract of *Inonotus obliquus*	Bioactive compounds	Inhibition of phosphorylation of Rb and E2F1 protein	Arresting the cell cycle and inhibiting the growth of CRC cells	(85)
Methylselenol	Selenium metabolite	Reduction in E2F1 protein levels	Arresting the cell cycle and inhibiting the *in vitro* growth of mouse CRC cells; decreasing the xenograft growth of mouse CRC cells	(86)
Irinotecan	Samptothecin derivative; topoisomerase I inhibitor	Decrease in E2F1 protein levels	Overcoming the resistance to 5-FU; inhibiting the xenograft growth of 5-FU resistant CRC cells in combination with S-1	(87)
Non-digestible fraction of beans	Extraction of beans	Downregulation of E2F1 mRNA	Contributing to the chemoprotective effect on early-stage CRC	(88)
SU9516	3-substituted indolinone compound; CDK2 inhibitor	Selective inhibition of cdk2 activity to prevent dissociation of pRb from E2F1	May mediate cell growth arrest	(89)
Ixocarpalactone A	Withanolide from *P. philadelphica*	Downregulation of E2F-1 and DP-1 proteins	Inducing cell cycle arrest, apoptosis, and formation of multiple vacuoles in CRC cells	(90)
RRR-α-Tocopherol succinate	Analogue of vitamin E	Downregulation of E2F-1 protein	Inducing apoptosis and inhibiting cell proliferation and colony formation in CRC cells	(91)
Tetrandrine	Bis-benzylisoquinoline alkaloid from *Stephania tetrandra*	Induction of proteasome-dependent degradation of E2F1	Inhibiting proliferation, arresting cell cycle, and inducing apoptosis in CRC cells	(92)

phosphorylation of Rb was further confirmed in zebrafish embryos [102]. Additionally, Evangelou *et al.* presented identical findings where E2F1 overexpression correlated with reduced proliferation and a better prognosis in colonic adenocarcinomas. However, the authors admitted a diverging result in oesophageal squamous-cell carcinomas (OSCCs), which positively correlated E2F1 with cancer progression. It was suspected that these conflicting results stemmed from the intestinal nature of the metaplastic Barrett mucosa [103]. Hao *et al.* found that E2F1-induced pro-apoptotic activity in CRC cells was regulated by PUMA upregulation and Bax translocation [104]. Another report indicated that E2F1 induced apoptosis of CRC cells through activating the transcription of SIVA1 apoptosis inducing factor (SIVA1) [105]. Although all of these inconsistent findings present a paradox, it is notable that they mostly occur after exogenous transfection of E2F1. In particular, our recent work showed that exogenous overexpression of E2F1 upregulated the levels of p73 and apoptotic peptidase activating factor 1 (Apaf1) and was accompanied with increased apoptosis in oxaliplatin-treated CRC cells, while endogenous E2F1 deacetylated by Sirt1 conferred the resistance in oxaliplatin-resistant CRC cells [52]. Thus, we suspect a mechanism in which exogenous E2F1 protein with different modifications activates different downstream genes as compared to its local endogenous interplay.

Conclusion and future perspectives

Undoubtedly, E2F1 is a major player in CRC, as it positively correlates with cancer development and poor prognosis. Downregulation of E2F1 has been shown to be helpful in reducing CRC burden. However, other studies have described an intriguing pro-apoptotic effect of E2F1. Although the pro-apoptotic behavior of E2F1 relies predominantly on exogenous administration of E2F1 *in vitro*, most of the *in vivo* studies have associated E2F1 with poor prognosis and development of CRC. It is likely that endogenous E2F1 neutralizes the anticipated pro-apoptotic property of E2F1 in a CRC microenvironment. Both the dual carcinogenic and pro-apoptotic effects of E2F1 represent an ongoing paradox for the scientific community. Although evidence has established an pro-tumorigenic property of E2F1, other opposing views cannot be ignored. In our opinion, future work on E2F1 in CRC should focus on the following questions:

1) How is the pro-apoptotic function of E2F1 in CRC prevented in the presence of its increased expression? 2) Does E2F1 behave differently in different kinds of cancer? 3) How do specific drug candidates against E2F1 inhibit the development of CRC?

Conclusively, E2F1 in CRC is a prospective drug target that currently lacks proper elucidation. Future research focused on answering the above questions may help to better understand the roles of E2F1 in CRC and may represent a key step in improving CRC therapeutics.

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Disclosure of conflict of interest

None.

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