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REVIEW

Regulation of *MYC* gene expression by aberrant Wnt/β-catenin signaling in colorectal cancer

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Abstract

The Wnt/β-catenin signaling pathway controls intestinal homeostasis and mutations in components of this pathway are prevalent in human colorectal cancers (CRCs). These mutations lead to inappropriate expression of genes controlled by Wnt responsive DNA elements (WREs). T-cell factor/Lymphoid enhancer factor transcription factors bind WREs and recruit the β-catenin transcriptional co-activator to activate target gene expression. Deregulated expression of the *c-MYC* proto-oncogene (*MYC*) by aberrant Wnt/β-catenin signaling drives colorectal carcinogenesis. In this review, we discuss the current literature pertaining to the identification and characterization of WREs that control oncogenic *MYC* expression in CRCs. A common theme has emerged whereby these WREs often map distally to the *MYC* genomic locus and control *MYC* gene expression through long-range chromatin loops with the *MYC* proximal promoter. We propose that by determining which of these WREs is critical for CRC pathogenesis, novel strategies can be developed to treat individuals suffering from this disease.

Key words: Wnt; β-catenin; Chromatin looping; Wnt responsive DNA element; MYC

Core tip: In colon cancer, mutations in components of the Wnt/β-catenin signaling pathway result in inappropriate *c-MYC* proto-oncogene (*MYC*) expression. To understand colorectal carcinogenesis requires the identification of Wnt responsive DNA elements (WREs) that control *MYC* expression in colorectal cancer (CRC). Through efforts to characterize *MYC* WREs, a model has emerged where several of these WREs appear largely dispensable for intestinal homeostasis, but are instead "hijacked" by oncogenic Wnt/β-catenin signaling to drive CRC. These findings raise the intriguing possibility that these WREs may be targeted therapeutically as an alternative approach to treat individuals afflicted by CRC. In this review, we summarize the literature describing the identification of *MYC* WREs and discuss how those involved in colorectal carcinogenesis may be targeted to limit progression of CRC.

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INTRODUCTION

The canonical Wnt/β-catenin signaling pathway regulates stem cell self-renewal, cell proliferation, and cell-fate decisions in the intestinal crypt microenvironment[1]. The β-catenin transcriptional co-activator governs the Wnt response, therefore, its distribution and levels within a cell are tightly regulated[2]. When cells, such as the differentiated cells in the apical regions of the intestinal crypts, are not exposed to Wnt, cytoplasmic β-catenin associates with a multi-protein "destruction complex"[3]. This complex contains adenomatous polyposis coli (APC), the axis inhibition proteins 1 and/or 2 (AXIN1/2), casein kinase 1 (CK1), and glycogen synthase kinase three beta (GSK3β). Here, APC and AXIN1/2 function as scaffolds to position β-catenin in proximity of CK1 and GSK3β. Phosphorylation of β-catenin by CK1 and GSK3β prime it for ubiquitination by the β-transducin-repeat-containing protein (β-TrCP) and subsequent degradation *via* the proteasome[4]. In the absence of Wnt, members of the T-cell factor/Lymphoid enhancer factor (TCF/Lef) family of sequence-specific transcription factors bound to Wnt responsive DNA elements (WREs) recruit transcriptional corepressor complexes. These complexes include transducin-like enhancer (TLE) and C-terminal binding protein, which associate with histone deacetylases to repress target gene expression (Figure 1A)[5].

The basal regions of the intestinal crypts contain stem cells and transit-amplifying progenitor cells[6]. These cells are exposed to Wnt ligand, secreted by the surrounding mesenchyme and differentiated Paneth cells, which binds frizzled (FZD)/low-density lipoprotein receptor-related protein 5 or 6 receptor complexes expressed on the cell surface[3]. This binding results in the subsequent recruitment of AXIN1/2 to the plasma membrane *via* interaction with dishevelled proteins (DVLs) and inactivation of the destruction complex. β-Catenin then escapes proteasomal degradation, accumulates in the cytoplasm, and translocates into the nucleus where it displaces TLE from TCF/Lef bound WREs[1]. β-Catenin/TCF/Lef complexes in turn recruit histone-modifying complexes, such as CBP/p300 protein acetyltransferases and MLL/Set methyltransferases, and chromatin remodeling complexes, including SWI/SNF, to induce expression of Wnt/β-catenin target genes and drive cellular proliferation (Figure 1B)[7].

Colorectal cancer (CRC) is the third leading cause of cancer related deaths in the United States. In 2015, 140000 individuals are predicted to be diagnosed with CRC and over 50000 patients are predicted to succumb to the disease[8]. Approximately 90% of sporadic CRCs contain mutations in components of the Wnt/β-catenin signaling pathway[9]. These mutations are found in the earliest neoplasms suggesting that this pathway serves as a critical gatekeeper to prevent colorectal carcinogenesis[10]. Most tumors contain a mutation in a single component of the pathway, although recent data from the cancer genome atlas consortium indicates that mutations in multiple components can co-occur[11]. The majority of mutations, some 85%, map to "hotspots" within APC and often lead to expression of a truncated APC protein from this allele[12]. This truncated protein is incapable of incorporating into a functional β-catenin destruction complex. Thus, when the wild-type APC is inactivated by mutation or lost through loss of heterozygosity, β-catenin levels inappropriately accumulate in the cell, leading to aberrant expression of Wnt/β-catenin target genes and the development of benign adenomas (Figure 1C)[1]. As these adenomas accumulate additional mutations in other signaling pathways, they transition into carcinomas[13]. Therefore, CRC is a disease of uncontrollable Wnt/β-catenin signaling where β-catenin/TCF complexes bound to WREs drive pathogenic expression of downstream target genes. To understand CRC initiation and progression requires identification of these genes and the WREs that control their expression.

The *c-MYC* proto-oncogene (*MYC*) was identified as a Wnt/β-catenin target gene using a differential RNA expression screen conducted in the human HT29 CRC cell line harboring mutant *APC* alleles[14]. MYC is a basic helix-loop-helix zipper (bHLHZ) transcription factor that heterodimerizes with bHLHZ factor MAX[15]. MYC: MAX heterodimers bind E-box sequence motifs to predominantly activate transcription of genes by recruiting histone modifying and chromatin remodeling complexes[16,17]. MYC: MAX regulates expression of thousands of target genes whose products control a wide-range of cellular processes including metabolism, ribosome biogenesis, and protein synthesis[18]. As such, MYC promotes cellular proliferation and cell growth[19].

MYC expression is deregulated in 50% of all cancers, including CRC[20]. In fact, *Myc* is required for tumorigenesis in mouse models of CRCs[21-25]. Given that deregulated *MYC* expression by oncogenic Wnt/β-catenin signaling is critical for colorectal carcinogenesis, several groups have sought to identify and characterize WREs that control its expression in human CRC cell lines over the years (Table 1). In this review, we summarize the literature pertaining to WREs that regulate *MYC* in CRC cells. We define a *MYC* WRE as a region of DNA that is: (1) bound by β-catenin/TCF complexes; (2) associated with histone modifications that demarcate enhancer elements, such as monomethylated lysine 4 on histone H3 (H3K4me1) and acetylated lysine 27 on histone H3 (H3K27Ac); (3) responsive to β-catenin/TCF complexes in luciferase reporter assays; and/or (4) juxtaposed to the *MYC* promoter region through chromatin loops, if it maps distal to the *MYC* gene. We then discuss

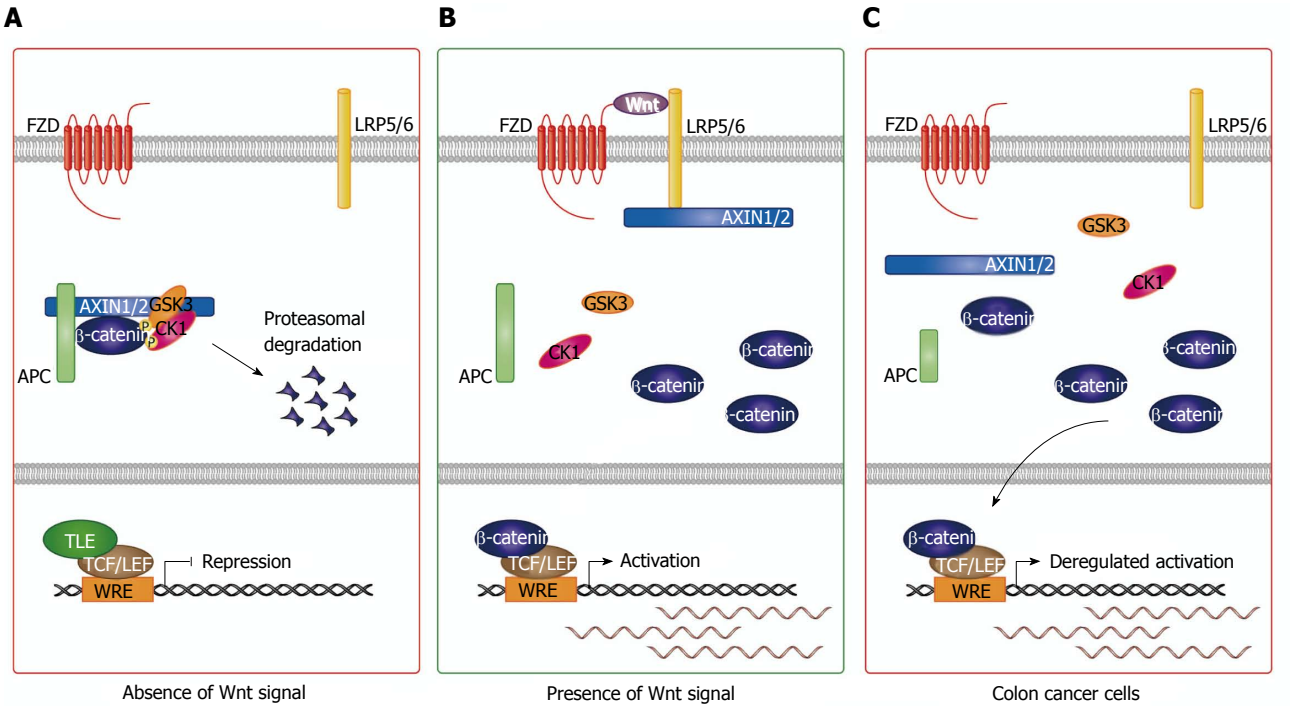

Figure 1 The Wnt/β-catenin signaling pathway. A: In the absence of a Wnt ligand, cytoplasmic β-catenin is targeted for proteasomal degradation by a multi-protein "destruction complex". Within the nucleus, TCF/Lef at WREs associates with the TLE co-repressor to repress Wnt/β-catenin target gene expression; B: Upon Wnt ligand binding to the FZD and LRP5/6 co-receptor complex, AXIN1/2 is recruited to the plasma membrane and the destruction complex is inactivated. β-Catenin subsequently translocates to the nucleus where it binds TCF/Lef assembled at WREs and recruits co-activator complexes to activate Wnt/β-catenin target gene expression; C: In CRCs, truncations of the APC protein prevent efficient targeting of β-catenin for proteasomal degradation. Therefore, nuclear β-catenin levels are elevated and aberrantly associate with TCF/Lef at WREs to drive deregulated expression of Wnt/β-catenin target genes. WRE: Wnt responsive DNA element; TCF/Lef: T-cell factor/Lymphoid enhancer factor; TLE: Transducin-like enhancer; FZD: Frizzled; LRP5/6: Lipoprotein receptor-related protein 5 or 6; AXIN1/2: Axis inhibition proteins 1 and/or 2; CRC: Colorectal cancer; APC: Adenomatous polyposis coli; GSK3: Glycogen synthase kinase three; CK1: Casein kinase 1.

Table 1 *MYC* Wnt responsive DNA element chromosomal locations

MYC WRE	Chromosomal position	Distance to *MYC* TSS	Ref.
MYC 5′ WREs	chr8: 128747425	889-bp upstream	[14,28]
MYC 3′ WRE	chr8: 128755419	7105-bp downstream	[26,27,31]
Myc 3′ WRE	chr15: 61992066	6725-bp downstream	[32,33]
MYC -335 WRE	chr8: 128413279	335036-bp upstream	[46-51,55,56]
Myc -335 WRE	chr15: 61450712	534630-bp upstream	[52]
MYC -520 WRE	chr8: 128227619	520696-bp upstream	[59,61]
MYC -517 WRE	chr8: 128231269	517046-bp upstream	[59]
MYC -513 WRE	chr8: 128235209	513106-bp upstream	[59,61]
MYC -488 WRE	chr8: 128259779	488536-bp upstream	[59,61]
MYC -470 WRE	chr8: 128278129	470186-bp upstream	[59,61]
MYC super-enhancer	chr8: 128275000	473314-bp upstream	[61,69]

TSS: Transcription start site; WRE: Wnt responsive DNA element.

future avenues of research aimed at determining which of these are the critical drivers for oncogenic *MYC* expression and how these results may be leveraged to develop new therapeutic options to manage individuals afflicted by CRC.

PROMOTER PROXIMAL *MYC* WREs

In the aforementioned study identifying *MYC* as a target of oncogenic Wnt/β-catenin signaling in human CRC cells, He *et al*[14] localized a β-catenin-responsive region within the *MYC* proximal promoter (Figure 2). This region contained two TCF binding elements, TBE1 and TBE2, which mediated β-catenin/TCF-responsiveness of this element in luciferase reporter assays. While expression of a dominant negative TCF4 protein lacking its amino-terminal β-catenin interaction domain decreased *MYC* expression in CRC cells, it was not evaluated at that time whether β-catenin/TCF complexes occupied this region at the endogenous *MYC* locus. However, this study identified the first *MYC* WRE and we have referred to it as the *MYC* 5′ WRE in our studies of WREs that control *MYC* expression in human CRC lines[26,27].

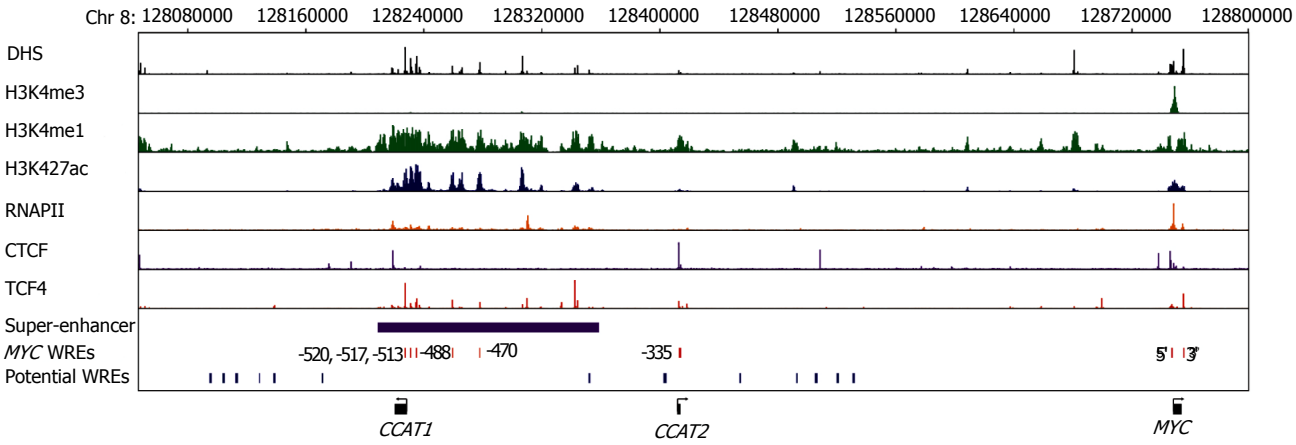

Figure 2 The *MYC* genomic locus in colorectal cancer. ChIP-Seq and DHS data in the HCT116 CRC cell line were downloaded from the WashU Epigenome Browser (http://epigenomegateway.wustl.edu/). The *MYC* distal super-enhancer is denoted as a purple rectangle, the *MYC* WREs discussed in this review are depicted as red lines, and potential WREs are denoted as blue lines[50,73]. WRE: Wnt responsive DNA element; CTCF: CCCTC-binding factor; H3K4me1: Monomethylated lysine 4 on histone H3; H3K27Ac: Acetylated lysine 27 on histone H3; RNAP: RNA Polymerase II; TCF: T-cell factor; CCAT: Colon-cancer associated transcript; DHS: DNase hypersensitivity.

Sierra *et al*[28] characterized a region mapping approximately 1200-bp upstream from the *MYC* transcriptional start site (TSS), and slightly upstream from *MYC* 5′ WRE, that harbors a third TBE, TBE3. In an extensive set of chromatin immunoprecipitation (ChIP) analyses, they found that β-catenin/TCF complex binding to this region correlated with *MYC* transcription in CRC cells. They also demonstrated that when Wnt signaling was shut-off, APC removed β-catenin and transcriptional co-activators from this site and replaced them with transcriptional corepressors to repress *MYC* expression. Interestingly, unlike wild-type APC, truncated APC found in CRC cells was unable to mediate this corepressor exchange, providing one mechanism underlying how APC mutations lead to aberrant *MYC* expression.

The identification and characterization of *MYC* promoter proximal WREs was an important step forward in our understanding of how β-catenin/TCF complexes drive *MYC* expression. However, very low levels of *MYC* transcripts are produced from a transgene in mice containing the human *MYC* genomic locus, and regions 2.3-kb upstream and 0.4-kb downstream, suggesting that additional WREs may contribute to regulating *MYC* expression[29].

THE DOWNSTREAM *MYC* PROXIMAL WRE

To identify regulatory elements that control *MYC* expression, Mautner *et al*[30] mapped DNaseI hypersensitivity sites in proximity to the *MYC* gene in the Colo320 CRC cell line. This strategy mapped a strong hypersensitivity site 1.5-kb downstream from the *MYC* transcription stop site, although it was not determined at that time whether this region demarcated a WRE. In a subsequent study, some 13 years later, a strong β-catenin binding region that overlapped this hypersensitive site was identified in the HCT116 human CRC cell line[31]. Using a combination of ChIP assays, luciferase reporter assays, and *MYC* transcript analysis, it was determined that this β-catenin/TCF binding element demarcated a WRE termed the *MYC* 3′ WRE (Figure 2)[26]. Using chromosome conformation capture (3C), it was demonstrated that β-catenin/TCF4 complexes coordinated a chromatin loop that integrated *MYC* 5′ and 3′ WREs in CRC cells[27]. This chromatin conformation was absent in quiescent CRC cells when *MYC* expression is silenced, but was induced by serum mitogens as *MYC* levels increased, indicating that loop formation accompanied *MYC* transcriptional activation. Interestingly, in HEK293 cells that contain an intact Wnt/β-catenin signaling pathway, either treatment with Wnt3A ligand or activation of the pathway with the GSK3 inhibitor lithium chloride (LiCl), failed to induce the chromatin loop even though *MYC* expression was increased. However, over-expression of oncogenic β-catenin (S45F) in these cells induced the *MYC* 5′3′ loop. Thus, the *MYC* 5′3′ chromatin conformation required elevated levels of nuclear β-catenin that typifies CRC.

In the colonic crypts of mice harboring a germ-line deletion of the *Myc* 3′ WRE, there was a 2-fold increase in *Myc* transcript levels and a 2.5-fold increase in MYC protein levels relative to levels seen in the colons of wild-type littermates[32]. This increase in MYC correlated with an increase in the number of proliferative cells and a decrease in the number of differentiated cells comprising the colonic crypt epithelium. Two approaches were taken to ascertain the role of this WRE in colorectal carcinogenesis[33]. First, *Myc* 3′ WRE$^{-/-}$ mice were bred to *Apc*$^{Min/+}$ mice that harbor a mutation in one *Apc* allele and spontaneously form intestinal tumors as the mice age and the second wild-type *Apc* allele is lost[34,35]. In comparison to *Apc*$^{Min/+}$, *Apc*$^{Min/+}$ *Myc* 3′ WRE$^{-/-}$ mice contained 4-fold more colonic tumors. Second, *Myc* 3′ WRE$^{-/-}$ mice and wild-type littermates were subjected to the azoxymethane/dextran sodium sulfate model of

colitis-associated colorectal cancer (CAC). *Myc* 3' WRE$^{-/-}$ mice subjected to this protocol contained an elevated number of tumors along the entire colonic tract, most notably in the ceca, which presented 20-fold more tumors. Thus, the primary function of this WRE is to repress *Myc* in the colonic epithelium.

DISTAL *MYC* WREs

The MYC -335 WRE

The *MYC* locus maps within a gene-poor region on chromosome 8q24[36]. Upstream of *MYC*, results from genome-wide association studies identified regions that harbor single nucleotide polymorphisms that are associated with an increased risk for the development of colon, breast, and prostate cancers[37-44]. One SNP in particular, rs6983267, has received interest in the field, with the G allele associated with increased risk of CRC over the T allele[40,42,44]. Despite this relationship, it was unknown how this SNP contributed to CRC, as *MYC*, the nearest protein-coding region, resides 335-kb downstream[45]. Using the enhancer element locator computational method, Tuupanen *et al*[46] found that a region encompassing rs6983267 was predicted to harbor a strong enhancer. Importantly, rs6983267 is adjacent to a TCF4 binding motif and was predicted to influence the binding of this factor. Indeed, the G allele conferred greater Wnt-responsiveness through this site that correlated with enhanced TCF4 binding. In an accompanying manuscript, Pomerantz *et al*[47] shed light on the mechanism for how the rs6983267 risk variant might influence *MYC* expression. They found that this *MYC* -335 WRE was physically juxtaposed to the *MYC* promoter region through a long-range chromatin loop. This result was confirmed by both Ahmadiyeh *et al*[48] and Jäger *et al*[49] in studies to identify regions of 8q24 that interact with the *MYC* -335 WRE by 3C and capture Hi-C, respectively. Furthermore, Sotelo *et al*[50] also reported evidence for this long-range interaction and found that overexpression of β-catenin and TCF4 increased the frequency of the association. However, a subsequent study found that while the SNP had no effect on the efficiency of chromatin looping, the risk-associated allele increased expression of the linked *MYC* allele[51]. Although the precise mechanistic details remain to be fully defined, these findings offer a potential explanation for how transcription factors bound to a distal regulatory element can drive oncogenic *MYC* expression in CRC.

In 2012, Sur *et al*[52] reported findings from a mouse model containing a germ-line deletion in the *Myc* -335 WRE. As was the case in *Myc* 3' WRE$^{-/-}$ intestines, deletion of the *Myc* -335 WRE did not cause gross phenotypic alterations of the intestinal architecture. Moreover, deletion of the *Myc* -335 WRE did not alter the cellular composition of the intestinal epithelium or influence expression of *Myc* in the duodenum. However, *Myc* expression in the colons of *Myc* -335 WRE$^{-/-}$ was decreased relative to levels seen in the colons of wild-type littermates. Importantly, when these mice were bred to *Apc*$^{Min/+}$ mice, deletion of the *Myc* -335 WRE led to a reduced number of tumors that formed in both the small intestines and colons. Thus, this element is a critical regulator of intestinal tumors that arise from pathogenic Wnt/β-catenin signaling.

As the *Apc*$^{Min/+}$ mouse is an important model for CRC, it is somewhat limited by the fact that tumors preferentially arise in the small intestine and not the colon[53]. A recent study suggests that this phenotype may be due to the fact that stem cells in the small intestine divide more rapidly than those in the colons and hence there is an increased chance of accumulating mutations in the small intestine[54]. In addition, tumors that arise in *Apc*$^{Min/+}$ mice are primarily adenomas, which do not progress to carcinomas because the mice become moribund[53]. Therefore, to determine whether the *MYC* -335 WRE is required in colorectal carcinomas requires deleting this regulatory element in a human CRC cell line. As part of an elegant study to functionally annotate genes whose expression is influenced by colon cancer risk SNPs, Yao *et al*[55] used clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) to delete the *MYC* -335 WRE in HCT116 human CRC cells. While *MYC* -335 WRE$^{-/-}$ cells expressed less *MYC* relative to parental cells, it was not reported whether this influenced the oncogenic properties of these cells.

In an intriguing study, Ling *et al*[56] offered an additional explanation for how the *MYC* -335 WRE influences *MYC*-driven colorectal carcinogenesis. They found that a long non-coding RNA, which they termed colon-cancer associated transcript two (*CCAT2*), is expressed from this region and contains the rs6983267 SNP. *CCAT2* expression is elevated in colon tumors relative to levels detected in adjacent and uninvolved colonic mucosa. Overexpression of *CCAT2* in HCT116 cells promoted tumorigenesis when these cells were implanted into immunocompromised mice, whereas *CCAT2* knockdown diminished the invasive capacity of the KM12SM CRC cell line. Moreover, *CCAT2* overexpression increased *MYC* expression in HCT116 cells, whereas *CCAT2* knockdown decreased *MYC* expression in these cells. *CCAT2* itself is a Wnt/β-catenin target gene, and it interacts directly with TCF4 to augment TCF4-dependent expression of Wnt/β-catenin target genes, including *MYC*. Further studies are necessary to fully understand how the rs6983267 SNP impinges upon *CCAT2* function.

In an effort to understand the function of rs6983267 in colorectal carcinogenesis, Kim *et al*[57] cloned seven lncRNAs derived from the 8q24.21 gene desert region. It was found that one of these lncRNAs, termed cancer-associated region long non-coding RNA number 5 (*CARLo5*), played an important role in driving CRC. *CARLo5* expression was elevated in CRC relative to expression in normal adjacent tissues, and knocking down *CARLo5* expression diminished CRC cell proliferation and growth of these cells as tumors in athymic nude mice. Interestingly, a region containing the

MYC-335 WRE was juxtaposed to the *CARLo5* promoter region to drive its expression in CRC cells.

The MYC distal super-enhancer

In 2010, Bottomly *et al*[58] reported results from a ChIP-Seq screen to localize β-catenin binding sites throughout the genome in the HCT116 human CRC cell line. This screen confirmed that β-catenin bound the *MYC* 5′ WRE, 3′ WRE, and -335 WRE in these cells. In addition, they noted that a cluster of β-catenin binding sites mapped to a region approximately 400-500-kb upstream from the *MYC* TSS. In a follow-up study, it was found that these regions bound TCF4, β-catenin, and RNA Polymerase Ⅱ (RNAP) and are demarcated by histones with modifications that typify enhancer regions including H3K4me1 and H3KAc[59,60]. Four of five of these distal β-catenin-bound regions formed long-range chromatin loops with the *MYC* proximal promoter region. Interestingly, these conformations were not only present in HCT116 cells, but also the non-CRC cell lines HEK293 and TIG-1, suggesting that β-catenin might use these pre-existing chromatin loops to activate *MYC* gene expression. Moreover, the interaction frequencies between two of these regions and the *MYC* proximal promoter were induced upon stimulation of the cells with serum mitogens.

Several years later, Hnisz *et al*[61] found that this distal cluster of *MYC* WREs overlapped with a super-enhancer. According to Whyte *et al*[62], super-enhancers are "…large clusters of transcriptional enhancers - formed by binding of high levels of master TFs (transcription factors) and Mediator coactivator - that drive expression of genes that define cell identity". These super-enhancers also bind high levels of the chromatin-associated protein bromodomain containing protein 4 (BRD4)[63]. Through its bromodomain, BRD4 associates with acetylated histones and recruits the positive transcription elongation factor (PTEF-b) to promote transcriptional elongation by RNAP[64,65]. BRD4 also interacts directly with the Mediator complex, and Mediator has been shown to play a role in chromatin looping[64-66]. JQ1 is a selective inhibitor of BRD4 activity that competes with BRD4 binding to acetylated substrates[67]. Interestingly, treatment of multiple myeloma cells with JQ1 led to a preferential loss of BRD4 occupancy at super-enhancers and repression of oncogene expression, including *MYC*[63,68]. A comparison of H3K27Ac patterns obtained from ChIP-Seq analysis in normal colonic mucosa and the HCT116 CRC cell line revealed that high levels of nucleosomes containing this modification map to the distal *MYC* super-enhancer in CRC cells[61]. By overlaying the ChIP-Seq profile for TCF4 binding in these cells, Hnisz *et al*[61] identified four putative WREs embedded within the distal super-enhancer that corresponded to four of the five *MYC* WREs identified previously in this region by Yochum[59]. Indeed, luciferase assays conducted with reporters containing these elements confirmed that they functioned as WREs[61].

As is the case for the *MYC* -335 WRE, a lncRNA termed colon cancer-associated transcript one (*CCAT1*), localizes within the *MYC* super-enhancer[69]. Two isoforms of *CCAT1* are expressed; a long form, *CCAT1-L*, which is 5200-bp in length, and a short form, *CCAT1-S*, which is 2600-bp in length. Both isoforms are expressed at higher levels in CRCs relative to levels in patient matched uninvolved colonic mucosa[69-71]. In contrast to *CCAT1-S*, which localizes to the cytoplasm, *CCAT1-L* localizes to the nucleus. *CCAT1-L* knockdown in CRC cells reduces *MYC* expression, while increased expression of *CCAT1-L* from its chromosomal locus enhances *MYC* expression. It was demonstrated that *CCAT1-L* is an important regulator of chromatin looping between *MYC* WREs and the *MYC* promoter. Interestingly, this lncRNA not only promoted chromatin looping between the *MYC* super-enhancer and the *MYC* promoter, but also between the *MYC* super-enhancer and the *MYC* -335 WRE. In addition, the CCCTC-binding factor (CTCF) was shown to play a role in mediating the interactions between distal *MYC* WREs and the *MYC* promoter. *CCAT1-L* interacted directly with CTCF and stabilized CTCF binding to distal WREs, providing a mechanism for *CCAT1-L*-mediated regulation of *MYC*.

ACTIVATION OF *MYC* EXPRESSION BY ONCOGENIC WNT/β-CATENIN SIGNALING

To summarize, mutations in components of the Wnt/β-catenin signaling pathway lead to aberrant *MYC* expression in CRCs through β-catenin/TCF transcription complexes bound to WREs. The *MYC* 3′ WRE, *MYC* -335 WRE, and distal super-enhancer are juxtaposed to the *MYC* 5′ WRE within the proximal promoter region through long-range chromatin loops (Figure 3)[27,47-51,59,69]. We therefore propose a model in which "hijacked" distal WREs align to the *MYC* proximal promoter and increase the local concentration of β-catenin/TCF transcription complexes to drive oncogenic *MYC* expression in CRC. Thus, the *MYC* proximal promoter serves as a "landing pad" to coordinate chromatin conformations at *MYC*. As the 3C technique represents an average interaction frequency across a population of cells, it is unknown whether these chromatin loops occur simultaneously at a single *MYC* allele[72]. It is also important to note that in several cases, these chromatin loops are not restricted to CRC cells and their formation does not depend on Wnt/β-catenin signaling[48,51,59]. Thus, the conformation itself, and not its formation, may poise the *MYC* locus to receive oncogenic signals. Finally, it is probable that additional WREs and other enhancer elements contribute to deregulated *MYC* expression in CRC cells[50,73].

CONCLUSION

Despite the identification of MYC over 30 years ago

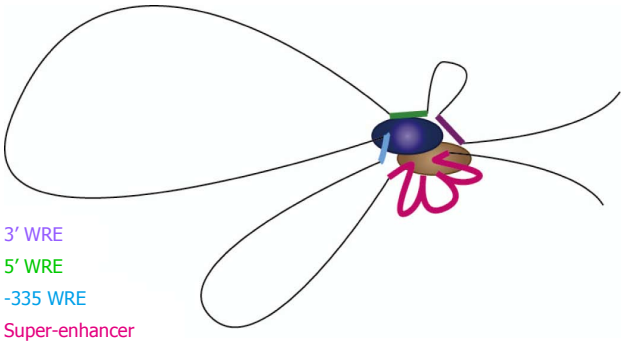

Figure 3 Model for the chromatin interaction network at the *MYC* gene locus in colorectal cancer cells with elevated nuclear β-catenin. β-Catenin "hijacks" MYC WREs in CRC cells, therefore, driving or stabilizing a distinct promoter-enhancer interaction network that is required for deregulated MYC gene expression. TCF/Lef and β-catenin are depicted as brown and purple ovals, respectively. WRE: Wnt responsive DNA element; CRC: Colorectal cancer; TCF/Lef: T-cell factor/Lymphoid enhancer factor.

and numerous reports linking deregulated *MYC* gene expression to tumorigenesis, there is still no clinically available drug that targets MYC in cancer cells[74-76]. This is in part due to the fact that, unlike other proteins, *MYC* is very rarely mutated in cancers[76]. Instead, *MYC* is overexpressed due to aberrant activation of upstream signaling pathways or due to events that trigger amplification of the *MYC* gene locus or insertions of activating sequences[77]. It is, therefore, difficult to target MYC in cancer cells *vs* normal, healthy cells where MYC is required for cellular proliferation[76]. Despite these difficulties, recent approaches to inhibit the BRD4 chromatin reader have been demonstrated to specifically and profoundly diminish *MYC* gene expression in several cancer cells[63,68,78-80]. These studies indicate that targeting *MYC* at the transcriptional level is an effective therapeutic strategy for treating cancers with deregulated *MYC* gene expression. Therefore, further characterization of the *MYC* WREs discussed in this review will likely provide new avenues for targeting *MYC* gene expression in CRC cells.

Recent reports have indicated that active DNA enhancer elements express short, bidirectional RNAs termed enhancer RNAs (eRNAs)[81-84]. Expression level changes observed for eRNAs correspond to changes in mRNA levels of the target gene, but the mechanism through which eRNAs activate target gene expression appears to vary based on cellular context. eRNAs have been found to stabilize enhancer-promoter interactions and also to relieve transcriptional pausing by inactivating the negative elongation factor complex[85,86]. It is unknown whether eRNAs are transcribed from the *MYC* WREs described in this report. It is feasible that eRNA transcripts derived from these *MYC* WREs facilitate *MYC* gene expression by stabilizing *MYC* WRE interactions with the *MYC* promoter. Additional factors could also be functioning to stabilize chromatin loops and activate *MYC* expression. β-Catenin was recently demonstrated to recruit cohesin and direct enhancer-promoter interactions in human embryonic stem cells[87]. If β-catenin also recruits cohesin at *MYC* WREs in CRC cells, it could explain how "hijacked" *MYC* WREs interact with the *MYC* promoter or suggest that β-catenin stabilizes pre-existing chromatin loops. CTCF could also be a critical factor for maintaining the genomic architecture at the *MYC* gene locus, as the interaction frequency of the *MYC* super-enhancer with the *MYC* promoter is diminished after CTCF knockdown[69]. To provide a better understanding of how β-catenin drives deregulated *MYC* gene expression in CRC cells, future work is needed to define the factors critical for *MYC* chromatin conformations and to determine the role of these conformations in activating *MYC* expression in CRCs.

Before candidate WREs can be considered as therapeutic targets, proof-of-principle experiments are required. Namely, whether these elements are dispensable in the normal colonic epithelium, but essential for colon carcinogenesis, which would suggest that a subset of WREs may be "hijacked" by oncogenic Wnt/β-catenin signaling. Indeed the mouse studies described by Sur *et al*[52] indicated that the *Myc* -335 WRE did not play a role in intestinal homeostasis, but was required for intestinal tumorigenesis caused by mutations in *Apc*. To determine whether this element was required in HCT116 human CRC cells, Yao *et al*[55] deleted it using CRISPR/Cas9 gene editing. While this deletion reduced *MYC* expression in these cells, it was not reported whether it altered the chromatin conformation at the *MYC* locus or reduced the oncogenic potential of these cells. Interestingly, the *MYC* distal super-enhancer is preferentially activated in CRC and not uninvolved colonic mucosa[61,88]. However, due to its size and complexity, it may be difficult to target using gene editing strategies. Although we reported that the *Myc* 3′ WRE suppressed colonic tumorigenesis in mice, we noted that deletion of this element decreased tumorigenesis in the small intestines of $Apc^{Min/+}$ mice[33]. Therefore, we used CRISPR/Cas9 to target the *MYC* 3′ WRE in HCT116 cells (SAR, GSY, unpublished). Using this approach, we generated clonal cell lines that harbored homozygous deletions in one of two TBEs within this element. These cells contain reduced *MYC* expression at the transcript and protein levels and also display reduced oncogenic properties.

Further investigation of the factors required for WRE-mediated transcriptional regulation of *MYC* will provide a more detailed model of how *MYC* gene expression becomes deregulated in CRCs. This model can then be applied to investigate deregulated *MYC* expression as a result of constitutive upstream signaling pathway activation in other cancers and also to identify potential therapeutic targets.

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