論文の内容の要旨

Title: Identification of FERM domain-containing protein 5 (FRMD5) as a novel target of β-catenin/TCF7L2 complex

（β-カテニン/ TCF7L2 複合体の新規標的遺伝子 FERM domain-containing protein 5 (FRMD5) の同定）

氏名： 朱 赤

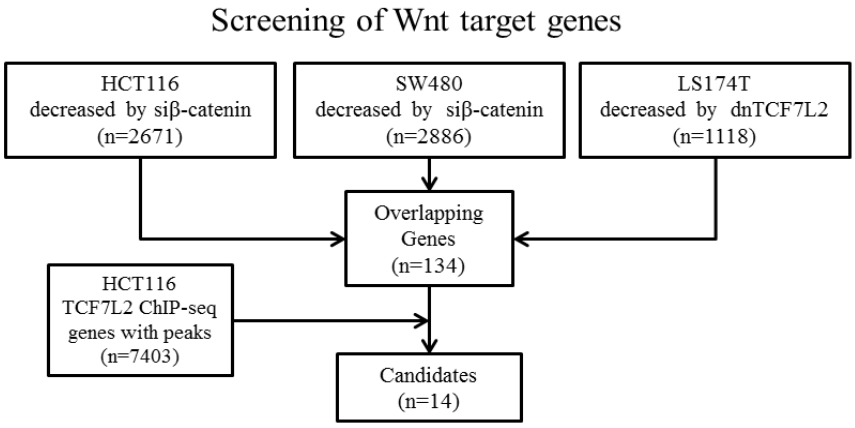
1. Background

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. Wnt/β-catenin pathway plays an important role in embryogenesis and the development and progression of tumors. In the great majority of human CRCs, frequent mutations in the components of the signaling pathway including APC and β-catenin are observed. These mutations induce abnormal accumulation of β-catenin, leading to the aberrant activation of canonical Wnt signaling. Accumulated β-catenin interacts with TCF7L2 and enhances its transcriptional activity, which results in the elevated expression of downstream target genes. Although a number of the downstream genes such as *cMYC* and *Cyclin D1* have been identified and their functional roles have been already well studied, the entire downstream players and their roles have not been clarified. In this study, I challenged to identify new downstream target genes of TCF7L2 and clarify their roles in the development and progression of colorectal tumors.

2. Results

2.1 Identification of downstream genes of β-catenin/TCF7L2

Expression profiles of HCT116 and SW480 cells treated with β-catenin or control siRNA were analyzed by my colleague in our laboratory. I additionally used public expression data of LS174T cells treated with dominant negative TCF7L2 (dnTCF7L2). As a result, I identified a total of 134 genes that were commonly down-regulated in response to the suppressed TCF7L2 activity by β-catenin siRNA or dnTCF7L2. To determine direct targets of β-catenin/TCF7L2 complex, I additionally performed chromatin immunoprecipitation sequencing (ChIP-seq) assay with anti-TCF7L2 antibody. Peak-calling process detected a total of 7403 peaks that have significantly increased read-counts compared with the background in the genome. Among the 134 candidate genes, 14 genes harbored at least one peak of the 7403 within a region between 10 kb upstream and downstream of the gene (Figure 1). Notably, the 14 genes included *AXIN2*, *RNF43*, and *MYC*, well-known direct targets of the complex.



Gene Symbol	Fold change in microarray data		
	HCT116 with β-catenin siRNA	SW480 with β-catenin siRNA	LS174T with dnTCF7L2
AXIN2[#]	-19.3	-4.8	-5.0
CDCA7L	-1.9	-1.6	-2.0
DPYSL3	-1.6	-1.5	-2.1
FRMD5	-2.7	-1.8	-2.0
JPH1	-2.5	-2.3	-1.7
MOSPD1	-2.0	-1.9	-1.7
MSI2	-5.3	-1.9	-1.8
MTBP	-2.2	-1.7	-1.7
MYC[#]	-1.5	-4.1	-2.3
OXR1	-1.8	-8.6	-1.6
PDE4D	-1.6	-3.7	-1.6
PHLDB2	-2.1	-2.1	-2.7
RNF43[#]	-5.4	-1.8	-2.7
SNTB1	-2.4	-2.2	-1.8

Figure 1. Fourteen candidate downstream genes of Wnt/β-catenin signaling in the colon cancer cells. Reported target genes, *AXIN2*, *RNF43* and *MYC*, are marked with "*".

2.2 *FRMD5* is a direct target of β-catenin/TCF7L2

In this study, I focused on *FRMD5* among the 13 genes. In agreement with the microarray data, quantitative PCR analysis confirmed that the expression of *FRMD5* was reduced by the knockdown of β-catenin by two different siRNAs in HCT116 and DLD1 cells (data not shown). In addition, according to the data of ONCOMINE, four of eight microarray data (TCGA Colorectal, Sabates-Bellver Colon, Gaedcke Colorectal and Hong Colorectal) showed significant increase of FRMD5 expression (≥ 2.0-fold) in CRC tissues compared with normal colonic tissues (p value <0.01).

2.3 Identification of a regulatory region of *FRMD5* by β-catenin/TCF7L2

To find candidate regulatory regions, I searched candidate regulatory regions from the data of histone modifications (high H3K4me1, high H4K27Ac and low H3K4me3) in the ENCODE. In the intron1 of *FRMD5*, I found a region with high H3K4me1 and high H4K27Ac between chr15:44449680 and chr15:44450487(hg19), suggesting an enhancer region (Figure 2). To analyze the regulatory activity of this region, I constructed reporter plasmids containing this putative binding region. As expected the reporter activity was decreased by the

knockdown of β-catenin. A search of transcription factor-binding motifs predicted six TCF7L2 binding elements (TBEs) in this region. Mutation in a TBE significantly reduced reporter activity in response to β-catenin siRNA, suggesting that β-catenin/TCF directly regulates expression of *FRMD5* through intron1 of *FRMD5*.

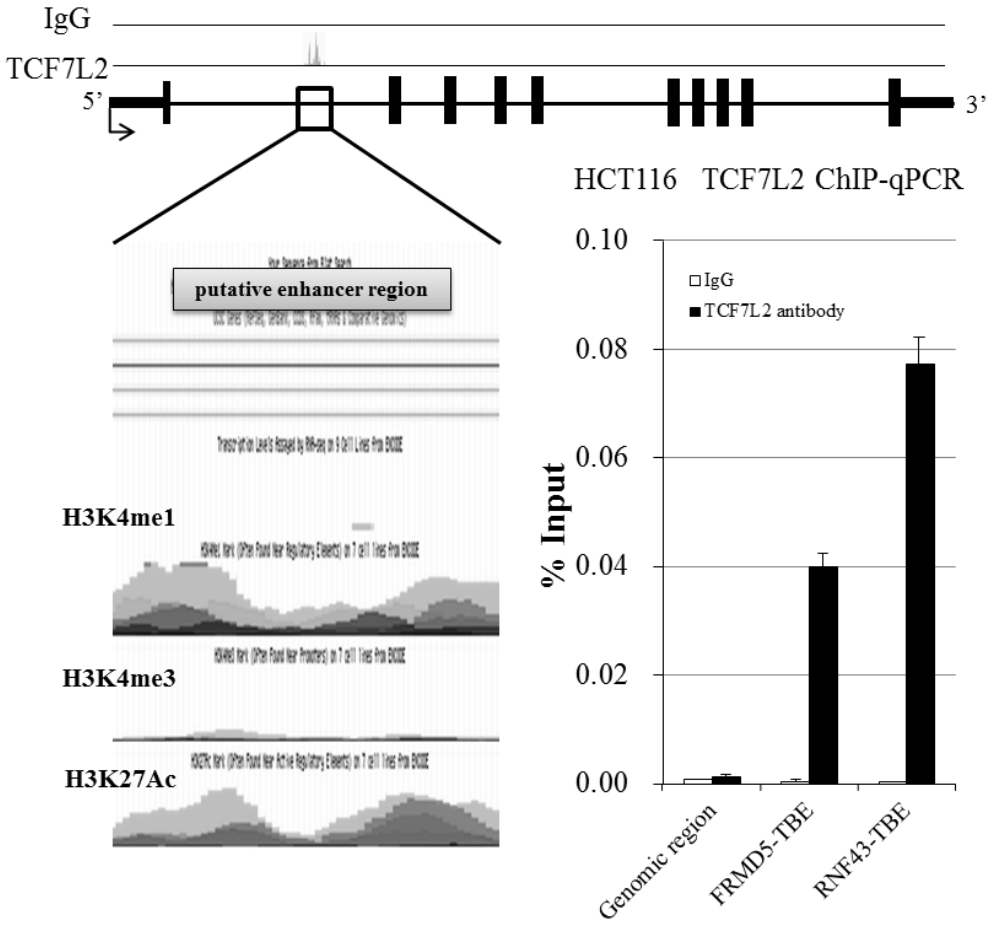


Figure 2. ENCODE data of histone modifications in *FRMD5* intron 1(left). ChIP-qPCR data of the candidate region (right). *RNF43* gene was used as control.

2.4 The role of FRMD5 as a downstream target of Wnt signaling.

To uncover the role of FRMD5 in CRC, we carried out expression profile analysis of HCT116 cells treated with/without FRMD5 siRNA. Among genes that were identified in microarray of FRMD5 knockdown, I further selected genes whose expression was also consistently altered by β-catenin siRNA in HCT116 cells to elucidate the function of FRMD5 as a downstream target of canonical Wnt signaling. Finally, a total of 36 and 53 genes that were either down-regulated or up-regulated, respectively, by FRMD5 siRNA as well as β-catenin siRNA were applied for gene set enrichment analysis. The analysis identified ten enriched gene sets such as DNA replication, cell cycle, and extracellular matrix (ECM).

3. Discussion

It is reported that FRMD5 is a protein that associates with p120-catenin (Wang et al, 2012). Interestingly reduced expression of FRMD5 caused decreased expression of E-cadherin and increased expression of vimentin in lung cancer cells. In addition, a recent paper showed that FRMD5 regulates cell-motility through integrin β5 and ROCK1 (Hu et al, 2014). These data suggested that FRMD5 may play a role in the suppression of tumor progression. Although a group showed that its expression was regulated by mutant $p53^{R273H}$ in U251 glioblastoma cells (Brázdová et al, 2009), its regulation by Wnt signaling pathway have never reported. Additionally, Expression profile analysis and subsequent gene enrichment analysis revealed that FRMD5 may be involved in regulating cell cycle and ECM-regulation in CRC. However, further studies are essential to clarify the role played by FRMD5 in colorectal tumorigenesis.