DEAD box protein DDX1 promotes colorectal tumorigenesis through transcriptional activation of the LGR5 gene

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INTRODUCTION

Colorectal cancer is one of the leading causes of tumor-related mortality in the world. Colorectal carcinogenesis is considered to be a long-term process involving multiple genetic alterations. The adenomatous polyposis coli (APC) gene on chromosome 5p, TP53 on 17p, and SMAD4 on 18q are potential tumor suppressor genes for colorectal carcinogenesis, while KRAS on 12p is an oncogene.¹ ² Matano et al (2015) established an in vitro human colorectal cancer model through introduction of APC, SMAD4, TP53 and KRAS mutations in the intestinal organoid culture system.³

DDX1, a member of the DEAD box RNA helicase family, plays a critical role in testicular tumors. However, it remains to be clarified whether DDX1 is involved in other types of malignant tumors such as colorectal cancer. We disrupted the DDX1 gene in a human colorectal cancer cell line LoVo using the CRISPR/Cas9-mediated gene-targeting system. DDX1-KO LoVo cells exhibited a much slower growth rate, produced fewer colonies in soft agar medium, and generated smaller solid tumors in nude mice than parental LoVo cells. Such phenotypes of the DDX1-KO cells were mostly reversed by exogenous expression of DDX1. These results indicate that DDX1 is required for tumorigenicity of colorectal cancer cells. In the DDX1-KO cells, the cancer stem cell marker genes LGR5, CD133, ALDH1 and SOX2 were markedly suppressed. Among them, expression of LGR5, which is essential for tumorigenicity of colorectal cancer cells, was restored in the DDX1-transfected DDX1-KO cells. Consistently, the DDX1-KO cells lost sphere-forming capacity in a DDX1-dependent fashion. Reporter and chromatin immunoprecipitation assays revealed that DDX1 directly bound to the −1837 to −1662 region of the enhancer/promoter region of the human LGR5 gene and enhanced its transcription in LoVo cells. Repression of LGR5 by DDX1 knockdown was observed in 2 other human colorectal cancer cell lines, Colo320 and SW837. These results suggest that LGR5 is a critical effector of DDX1 in colorectal cancer cells. The DDX1-LGR5 axis could be a new drug target for this type of malignant cancer.

KEYWORDS

cancer stem cells, colorectal cancer, LGR5, RNA helicase, Tumorigenesis
Aberrant activation of the Wnt signaling pathway is a main oncogenic driver in 90% of colorectal cancer patients with APC mutations. In normal mucosa, the β-catenin level is kept low in the cytoplasm by the action of a destruction complex composed of glycogen synthase kinase 3, Axin1, casein kinase 1, APC and other factors. Mutations in APC abolish the destructive function, leading to the accumulation and nuclear translocation of β-catenin and subsequent transcriptional activation of its target genes, including LGR5, c-Myc and Cyclin-D1.

LGR5, also known as GPR49, is a member of the G protein-coupled receptor (GPCR) family. LGR5 was originally identified as a Wnt/Tcf4 target gene in colorectal cancer. LGR5 is overexpressed in colorectal, ovarian, hepatocellular and basal cell cancers. LGR5 expression was detected in human colorectal stem cells located between Paneth cells in the intestinal crypts. Furthermore, cell lineage-tracing experiments demonstrated that LGR5-positive cells are intestinal cancer stem cells (CSC). LGR5-positive intestinal stem cells are the cells of origin for adenoma caused by APC deletion and are present inside colorectal tumors in an Apc-KO mouse model.

DDX1 is a member of the DEAD box RNA helicase family characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD). DDX1 was originally identified by differential screening of a cDNA library enriched in transcripts present in a retinoblastoma cell line. DDX1 is co-amplified with MYCN and overexpressed in a subset of neuroblastoma and retinoblastoma cell lines and tumors. DDX1 is involved in a variety of biological processes, including trna synthesis, mRNA and microRNA processing, ribosome biogenesis, DNA repair, and nuclear factor-kappaB-mediated gene induction. Because DDX1 deficiency in mice causes early embryonic lethality, it must play essential roles in normal cells.

DDX1 plays a critical role in testicular tumorigenesis in part by promoting transcription of Cyclin-D2 and stem cell-related genes on human chromosome 12p. The expression level of DDX1 is elevated not only in germ cell tumors but also in retinoblastoma, neuroblastoma, glioblastoma and breast cancer. However, it remains unknown whether DDX1 plays a role in colorectal carcinogenesis.

In this study, we explored the function of DDX1 in human colorectal cancers by disrupting the DDX1 gene in a representative cell line LoVo. We showed that DDX1-KO LoVo cells have defects in colony and sphere-forming capacity in vitro and in vivo tumorigenesis in nude mice. More importantly, we demonstrated that DDX1 promotes the expression of the LGR5 gene by direct interaction with its enhancer/promoter region. Thus, DDX1 is an important regulator of colorectal CSC.

2 MATERIALS AND METHODS

2.1 Cell culture

LoVo, Colo320 and SW837 were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in DMEM (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin-streptomycin (PS; Sigma) at 37°C in humidified air with 5% CO2.

2.2 Gene disruption, overexpression and knockdown

Guide RNA (gRNA) sequence for the DDX1 gene was chosen using the clustered regularly interspersed short palindromic repeat (CRISPR) Direct tool (http://crispr.dbcls.jp/). Oligodeoxynucleotide encoding DDX1 single guide RNA (sgRNA) was inserted into the PX458 expression vector (Addgene, Cambridge, MA, USA), which bicistronically expresses sgRNA and the CRISPR-associated protein 9 (Cas9) nuclease. This was transfected into LoVo cells with Lipofectamine3000 (Thermo Fisher Scientific). After 48 hours in culture, GFP-positive cells were separated by FACS on a FACSAriaIII (BD Biosciences, San Jose, CA, USA) and cloned using steel cylinders. A DDX1-overexpressing LoVo cell clone was established using the retroviral vector pMY-IG as previously described. Retroviral introduction of siRNA for DDX1 gene was done as previously described.

2.3 Quantitative RT-PCR

Total RNA was extracted using TRIzol (Invitrogen, Paisley, UK). Synthesis of cDNA and quantitative PCR were carried out using PrimeScript RT Reagent Kit (Takara, Otsu, Japan), Thunderbird SYBR RT-PCR kit (Toyobo, Osaka, Japan), and a LightCycler480 System (Roche Applied Science, Indianapolis, IN, USA), according to the manufacturers’ protocols. GAPDH was used as an internal control. All reactions were carried out in triplicate. Sequence information of primers is shown in Table 1.

| Gene     | Forward primer                          | Reverse primer                        | Product (bp) |
|----------|-----------------------------------------|---------------------------------------|--------------|
| DDX1     | 5’- CAGAACCGATGTCCTCGCAGA -3’           | 5’- GAGCCCAATCCATCTCTTCCA -3’         | 287          |
| LGR5     | 5’- TCCAACCTCCGCTCGTCCAC -3’            | 5’- CGGAAAGCGTGAACCTCTCTCCTCA -3’     | 111          |
| CD133    | 5’- GAGTGGAAACATGGGACAGATAGCA -3’       | 5’- ACGCCTTGTGCTCTGTGAGTGG -3’        | 113          |
| ALDH1    | 5’- AGGCTTCCACGAGATACGAGA -3’           | 5’- GTGGCAGCAGCAGATACAC -3’           | 124          |
| SOX2     | 5’- TGAGCGCCCTGCACTACAA -3’             | 5’- GCTGGAGGACAGACATGTCTGAG -3’       | 84           |
| GAPDH    | 5’- GATCATCAGCAGATGCTCTCT -3’           | 5’- TTCAGCTCAGGATGACCTT -3’           | 240          |
2.4 Western blot analysis

Protein concentration was determined using the BCA Protein Assay Reagent (Thermo Fisher Scientific), and equal amounts of protein were subjected to 10% SDS-PAGE. Standard western blot analyses were performed using anti-DDX1 (11357-1-AP, ProteinTech, Chicago, IL, USA), anti-LGR5 (ab75732, Abcam, Cambridge, UK) or anti-α-tubulin (T9026, Sigma-Aldrich, St Louis, MO, USA) antibody. Specific protein bands were detected by HRP-conjugated secondary anti-rabbit or anti-mouse antibody (#7074, #7076, Cell Signaling, Danvers, MA, USA) and visualized with Image Quant LAS 1000 (GE Healthcare, Buckinghamshire, UK).

2.5 Xenograft experiments

Male athymic BALB/c nu/nu male mice (5 weeks old) were purchased from Nihon SLIC (Hamamatsu, Japan). LoVo cells (5 × 10⁶) in 100 μL of 50% Matrigel (BD Biosciences) were subcutaneously injected into nude mice. Tumor size was measured with a caliper every 7 days, and the estimated volume of the tumor was calculated using the following formula: volume = π/6 × (length × width²). All mice were maintained under a 12 hours light/12 hours dark cycle in a pathogen-free animal facility. All experimental procedures involving mice were preapproved by the Ethical Committee for Animal Experiments at Tokyo Metropolitan Institute of Medical Science and were performed according to the Guidelines for the Proper Conduct of Animal Experiments.

2.6 Sphere formation assay

LoVo cells (4 × 10³) were plated onto a 35 mm ultralow attachment plate (Greiner Bio-One, Frickenhausen, Germany) in 10% FBS-DMEM, supplemented with 50 μL of HA-Matrix (Agcell, Tottori, Japan). After 6 days in culture, the number of spheres (volume > 1.2 × 10⁴ μm³) was counted and their volume was analyzed on a BX-X700 fluorescence microscope (Keyence, Osaka, Japan). This experiment was performed 3 times in triplicate.

2.7 Luciferase reporter assay

A genomic DNA sequence containing the human LGR5 gene was obtained from a public database (Ensembl Gene ID: ENSG00000139292). DNA fragments covering the enhancer/promoter region of the LGR5 gene were PCR amplified using the genomic DNA of TIG-1 cells as a template and cloned into a pGL3 basic vector (Promega, Madison, WI, USA) using an In-Fusion HD Cloning Kit (Takara). LoVo cells (8 × 10⁵ cells/well) in 96-well white-bottom plates (Sumitomo Bakelite, Tokyo, Japan) were transfected with each reporter construct (200 ng) in combination with the pRL-CMV vector (Promega) using Lipofectamine 3000 reagent. In some experiments, the DDX1 expression vector (pMY-IG-DDX1) or the empty vector (pMY-IG) was cotransfected. After 48 hours in culture, enzymatic activities were analyzed using the Dual-Glo Luciferase reporter assay kit (Promega) in a Mithras LB 940 luminometer (Berthold Technologies, Bad Wildbad, Germany). Results were obtained from 2 independent experiments performed in triplicate.

2.8 Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed paraffin-embedded specimens, purchased from US Biomax (colorectal cancer tissue array, CO484a, Rockville, MD, USA). After de-waxing in xylene and graded ethanol, the section was incubated in 3% H₂O₂ solution to block endogenous peroxidase activity. The section was incubated with anti-DDX1 primary antibody (1:100) at 4°C overnight and processed using the DAB system (Vector, Burlingame, CA, USA). Paraffin sections of xenograft-derived tumors were stained with anti-PCNA antibody (1:100, M0879, Agilent, Santa Clara, CA, USA) in combination with AlexaFluor 546-conjugated anti-mouse IgG antibody (A11030, Thermo Fisher Scientific).

2.9 Immunofluorescence analysis

Cells were fixed with 2% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were incubated with anti-DDX1 or anti-LGR5 (ab75732, Abcam) antibody overnight at 4°C. They were stained with AlexaFluor 488-conjugated anti-rabbit IgG antibody (R37116, Thermo Fisher Scientific) and analyzed on a TSC SP8 Confocal microscope (Leica Microsystems, Mannheim, Germany).

2.10 ChIP assay

A cross-linked chromatin fraction of LoVo cells was subjected to immunoprecipitation using anti-DDX1 or anti-rabbit IgG antibody (ab172730, Abcam) as previously described. The amount of target genomic DNA in the immunoprecipitate was examined by real-time PCR on a LightCycler480. Sequence information of primers is shown in Table 2.

2.11 Statistical analysis

All statistical analyses were performed using Microsoft Excel. Differences between individual groups were analyzed using a 2-tailed unpaired t test. A P-value of <.05 was considered significant.

| Site    | Forward primer | Reverse primer | Product (bp) |
|---------|----------------|----------------|--------------|
| LGR5-P1 | 5'- CAGAGTGTTAAACGGATTTT -3' | 5'- AGTGCACTCATCACCAGATT -3' | 112 |
| LGR5-P2 | 5'- GCCCACGCTGTTAATCACTCC -3' | 5'- TTCAACATGTGACCTGAGTC -3' | 179 |
3 | RESULTS

3.1 | DDX1 is highly expressed in human colorectal cancer cell lines and tissues

We previously reported that DDX1 plays a critical role in testicular tumorigenesis using the human testicular germ cell tumor cell line, NEC8.\(^{20}\) To examine whether DDX1 is also involved in colorectal carcinogenesis, we first compared the mRNA expression level of DDX1 in NEC8 cells with that in 5 human colorectal cancer cell lines by quantitative RT-PCR. DDX1 was highly expressed in all of these colorectal cancer cell lines, with expression as high as that in NEC8, and much higher than that in normal mouse colorectal tissue (Figure 1A). In LoVo cells, DDX1 protein was localized in both nucleus and cytoplasm (Figure 1B). Next, we investigated the expression of DDX1 protein in various histological subgroups of human colorectal adenocarcinomas. Scale bars = 25 μm. C. Immunohistochemical staining of paraffin-embedded sections of normal human colon and various stages of human colorectal adenocarcinomas. Scale bars = 50 μm. TNM grading of human colon cancers is as follows. M0, no distant metastasis; M1, distant metastasis; N0, no regional lymph node metastasis; N1, metastasis in 1-3 regional lymph nodes; N2, metastasis in 4 or more regional lymph nodes; T3, tumor invades through muscularis propria into subserosa or into nonperitonealized pericolic or perirectal tissues; T4, tumor directly invades other organs or structures and/or perforate visceral peritoneum.
colorectal cancer and normal colon. Strong signals for DDX1 were detected in these colorectal cancer patient specimens, whereas they were below the detection level in normal colorectal tissue (Figure 1C). The amount of DDX1 protein did not correlate with the pathological grade and clinical stage of the colorectal cancer.

3.2 | DDX1 is required for proliferation of the human colorectal cancer cell line LoVo

To examine whether DDX1 plays an essential role in colorectal carcinogenesis, we disrupted the DDX1 gene of LoVo cells using CRISPR/Cas9-mediated genome editing. We cotransfected 2 gRNAs targeting the transcription start site (TSS) into LoVo cells and sorted GFP-positive cells by FACS. Through molecular characterization of DDX1 genes in several cell clones, we established a DDX1 KO LoVo cell line carrying a 106-bp biallelic deletion (~107 to ~2 relative to the TSS) in the 5′ untranscribed region of the DDX1 gene. DDX1 KO LoVo cells did not produce DDX1 mRNA or protein (Figure 2A). Morphologically, DDX1 KO LoVo cells had a more flattened shape with more vacuoles than parental WT LoVo cells (Figure 2C). Importantly, the growth rate of DDX1 KO cells in regular growth medium was approximately half that of WT cells (Figure 2D).

To confirm that the above phenotypic changes in DDX1 KO LoVo cells were caused by the lack of DDX1, we infected DDX1 KO cells with a DDX1 retroviral vector (pMY-IG-DDX1) to generate DDX1 KO + DDX1 LoVo cells. The mRNA expression level of DDX1 in DDX1 KO + DDX1 cells was fourfold higher than in WT cells (Figure 2A). Accordingly, the protein level of DDX1 in DDX1 KO + DDX1 cells was higher than in WT cells (Figure 2B). DDX1 KO + DDX1 cells were morphologically indistinguishable from WT cells (Figure 2C). Most importantly, the growth rate of DDX1 KO + DDX1 cells was faster than that of DDX1 KO cells and similar to that of WT cells in 8-day culture (Figure 2D).

We next compared cell cycle status (Figure 2E). The percentage of cells in G1 phase was significantly higher in DDX1 KO cells (54.6% ± 1.1%) than in WT cells (47.4% ± 0.9%) and DDX1 KO + DDX1 cells (46.7% ± 0.7%). Reflecting this fact, the relative frequency of cells in S phase was lower in DDX1 KO cells (31.2% ± 2.3%) than in WT cells (35.1% ± 3.3%) and DDX1 KO + DDX1 cells (34.5% ± 2.0%). These data suggested that the decreased proliferation of DDX1 KO cells is due to cell cycle arrest.

3.3 | DDX1 is required for density-independent and anchorage-independent growth of LoVo cells

We next compared growth capacity of WT, DDX1 KO and DDX1 KO + DDX1 LoVo cells under low cell density culture conditions. WT and DDX1 KO + DDX1 cells grew to form cell islands, whereas DDX1 KO cells produced fewer and much smaller cell islands (Figure 3A). The average size of DDX1 KO-derived cell islands was 16.3% that of WT cells, whereas that of DDX1 KO + DDX1-derived cell islands was 75.0% that of WT cells (Figure 3A).

Similar results were obtained in the soft agar colony formation assay. The average number of DDX1 KO-derived colonies was 28.1% that of the WT, whereas the number of DDX1 KO + DDX1-derived colonies was 79.4% that of the WT (Figure 3B). In addition, DDX1 KO-derived colonies in soft agar medium were smaller than WT-derived and DDX1 KO + DDX1-derived colonies (Figure 3B).
These results indicated that DDX1 is required for the clonogenic proliferation of LoVo cells.

### 3.4 DDX1 influences the in vivo tumorigenic capacity of LoVo cells

We next investigated a DDX1 function in tumorigenesis by employing the standard xenograft tumor mode. WT and DDX1-KO + DDX1 LoVo cells formed solid tumors of similar size under the skin of nude mice (Figure 4A-C). By contrast, the average size of DDX1-KO-derived tumors was nearly half that of those derived from WT and DDX1-KO + DDX1 (Figure 4A-C). H&E staining of the tumor sections revealed relative uniformity of the nucleus size of LoVo-derived cells (Figure 4D). Consistent with the smaller tumor size, PCNA-positive cells in the DDX1-KO + DDX1-derived tumor section were greatly decreased (Figure 4D). These results demonstrate that DDX1 plays important roles in colorectal cancer progression.

### 3.5 DDX1 is required for LGR5 expression in LoVo cells

Because we previously found that DDX1 is a transcriptional activator of stem cell-related genes in human testicular tumor cells, we next examined the expression of colorectal stem cell marker genes. In DDX1-KO LoVo cells, mRNA levels of LGR5, CD133, ALDH1 and SOX2 genes were <10% of those in WT LoVo cells (Figure 5A). Moreover, they were mostly (LGR5) or partially (CD133, ALDH1 and SOX2) restored by the exogenous DDX1...
expression in DDX1-KO + DDX1 LoVo cells (Figure 5A). Expression of LGR5 mRNA was repressed by siRNA-mediated knockdown of DDX1 in 2 other human colorectal cancer cell lines, Colo320 and SW837 cells (Figure 5B), excluding the possibility that this phenomenon is specific to LoVo cell line. In LoVo cells, loss of LGR5 by the DDX1 disruption and its rescue by exogenous DDX1 were detected at protein levels by western blotting (Figure 5C). Quantification of the immunofluorescent intensity of LGR5 protein in the cells also revealed such a difference between WT or DDX1-KO + DDX1 and DDX1-KO cells (Figure 5D). These results demonstrated that DDX1 is indispensable for the expression of LGR5 in LoVo cells.

LGR5-expressing cells represent colorectal CSC. To investigate the possibility that DDX1 plays a role in the maintenance of CSC, we carried out the tumor sphere formation assay. While WT LoVo cells generated approximately 240 spheres per 1000 cells, DDX1-KO LoVo cells produced eightfold fewer spheres (Figure 5E). The impaired sphere-forming capacity of DDX1-KO cells was partially restored by exogenous DDX1 expression in DDX1-KO + DDX1 cells (Figure 5E). The average volume of DDX1-KO-derived spheres also decreased to 24.3% that of WT-derived spheres. It was rescued to 45.3% by exogenous DDX1 expression in DDX1-KO + DDX1 cells (Figure 5E). These data suggest that DDX1 contributes to the sphere-forming capacity of a CSC population in LoVo cells.
3.6 DDX1 is a transcriptional activator of the LGR5 gene in LoVo cells

DDX1 directly binds to the enhancer/promoter region of the cyclin-D2 gene, thereby promoting its transcription. To clarify the underlying mechanism of the DDX1-dependent expression of the LGR5 gene in LoVo cells, we generated a series of luciferase reporter constructs carrying DNA fragments of the human LGR5 enhancer/promoter region (Figure 6A). When transfected into WT and DDX1-KO LoVo cells, LGR5-2937 and LGR5-1837 reporters displayed significantly higher values in WT cells than in DDX1-KO cells (Figure 6B). LGR5-1662, LGR5-1440 and LGR5-635 reporters showed no differences. We found that partial sequence for the DDX1-binding motif (5′-ACGTACACCTTTATGCC-3′) is present at 3 sites.
between −2937 and −1837, and 2 sites between −1837 and −1662 (Figure 6A). Importantly, mutation of AAGGTTGTG (−1680 and −1672) to TTGGGACTG in the LGR5-1837 reporter abrogated the significant activity difference between WT and DDX1-KO LoVo cells (Figure 6B). Next, we transiently transfected the LGR5-1837, 1837 mutant, 1662, 1440 or basic pGL3 reporter into WT LoVo cells in combination with the DDX1 expression vector (+DDX1) or the empty vector (+Empty). Luciferase activities of cell lysates were measured 48 h after transfection, and firefly vs Renilla (F/R) was calculated. C, WT LoVo cells were transfected with the indicated reporter constructs in combination with the DDX1 expression vector (+DDX1) or the empty vector (+Empty). Luciferase activities of cell lysates were measured 48 h after transfection, and firefly vs Renilla (F/R) was calculated. D, WT and DDX1-KO + DDX1 LoVo cells were subjected to ChIP assays using anti-DDX1 or control rabbit IgG. Relative abundance of the LGR5 gene-specific PCR product is shown. B–D, Each value represents the mean (n = 5) ± SD. **P < .01

4 | DISCUSSION

Here, we propose a critical function for DDX1 in colorectal carcinogenesis using the LoVo cell line as a model. We first showed that DDX1 mRNA is abundantly expressed in several human colorectal cancer cell lines. Previously, we reported the elevated expression of DDX1 in both seminoma and nonseminoma types of human testicular germ cell tumors. Amplification and/or overexpression of the DDX1 gene has been described in retinoblastoma, neuroblastoma, glioblastoma and breast cancer. Furthermore, according
to the Catalogue of Somatic Mutations in Cancer (COSMIC, Sanger Institute), certain percentages of cancer tissues are DDX1-positive: 12.6% of liver-derived, 14.7% of lung-derived, 16.9% of ovary-derived, 12.5% of cervix-derived, 7.9% of colorectal-derived, 7.7% of breast-derived and 6.5% of kidney-derived carcinomas. Although point mutations and copy number variations of the DDX1 gene were barely reported in any cases, the expression level of DDX1 could be positively correlated with carcinogenesis.

Next, we disrupted the DDX1 gene in LoVo cells using CRISPR/Cas9 technology. DDX1-KO LoVo cells displayed significantly less proliferation, anchorag e-independent colony formation and in vivo tumorigenesis than WT LoVo cells. These phenotypic changes were rescued by exogenous DDX1. These results indicate that DDX1 controls the tumorigenic capacity of colorectal cancer cells.

In DDX1-KO LoVo cells, expression of LGR5, CD133, ALDH1 and SOX2 genes was suppressed. These are colorectal CSC markers. Among them, LGR5 expression was fully restored by a high level of exogenously expressed DDX1. Expression of LGR5 was also repressed by DDX1 knockdown in Colo320 and SW837 cell lines. Subsequently, we found that DDX1 directly binds to the enhancer/promoter region of the human LGR5 gene and promotes its transcription. As is the case with DDX1, LGR5 is known to be required for proliferation, colony formation and tumor formation of colorectal cancer cells. Disruption of the LGR5 gene in colorectal and other cancer cell lines results in the loss of tumorigenicity. Importantly, LGR5-positive cells act as intestinal stem cells as well as CSC in colon. Sphere-forming capacity is one of the reliable properties of CSC. We showed that DDX1-KO LoVo cells produced fewer and smaller spheres than WT and DDX1-KO + DDX1 LoVo cells. LGR5 knockdown CSC display lower proliferation and sphere formation capacity. Expression levels of DDX1 in colorectal tumor sections were not positively correlated with histological grade. Consistently, levels of LGR5 in primary colorectal cancers are not correlated with clinicopathological features. Expression of LGR5 increases at early stages of colorectal tumorigenesis, and LGR5-positive cells are localized in the crypt base and invasive front areas. Taken together, we propose that DDX1 regulates tumorigenic capacity and malignancy of colon cancer cells through the transcriptional regulation of the LGR5 gene in CSC.

The transcription factor GATA6 enhances the expression of LGR5 in colorectal cancer cells by direct binding to its enhancer/promoter region. In addition, GATA6 knockdown abrogates the tumorigenic capacity of colorectal cancer cells. In the enhancer/promoter region of the LGR5 gene, binding sites for GATA6 and DDX1 were distinct, suggesting that DDX1 is a second critical regulator of the LGR5 gene in colorectal cancer cells. In summary, DDX1 plays important roles in vitro colony formation and in vivo tumorigenesis of the human colorectal cancer cell line LoVo. DDX1 is involved in the transcriptional control of the LGR5 gene, which is a functionally important CSC marker. Therefore, the DDX1-LGR5 axis is a new therapeutic target for colorectal cancers.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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