Significance of regenerating islet-derived type IV gene expression in gastroenterological cancers

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Abstract
The regenerating islet-derived members (Reg), a group of small secretory proteins, which are involved in cell proliferation or differentiation in digestive organs, are upregulated in several gastrointestinal cancers, functioning as trophic or antiapoptotic factors. Regenerating islet-derived type IV (RegIV), a member of the Reg gene family, has been reported to be overexpressed in gastroenterological cancers. RegIV overexpression in tumor cells has been associated with carcinogenesis, cell growth, survival and resistance to apoptosis. Cancer tissue expressing RegIV is generally associated with more malignant characteristics than that without such expression, and RegIV is considered a novel prognostic factor as well as diagnostic marker in some gastroenterological cancers. We previously investigated the expression levels of RegIV mRNA of 202 surgical colorectal cancer specimens with quantitative real-time reverse-transcriptase polymerase chain reaction and reported that a higher level of RegIV gene expression was a significant independent predictor of colorectal cancer. The biologic functions of RegIV protein in cancer tissue, associated with carcinogenesis, anti-apoptosis and invasiveness, are being elucidated by molecular investigations using transfection techniques or neutralizing antibodies of RegIV, and the feasibility of antibody therapy targeting RegIV is being assessed. These studies may lead to novel therapeutic strategies for gastroenterological cancers expressing RegIV. This review article summarizes the current information related to biological functions as well as clinical importance of RegIV gene to clarify the significance of RegIV expression in gastroenterological cancers.

Key words: Regenerating islet-derived type IV protein; Gastrointestinal neoplasms; Prognosis; Epidermal growth factor receptor/protein kinase B

INTRODUCTION
It is generally accepted that cancer develops as a result of multiple genetic alterations. A better understanding of the changes in gene expression that occur during carcinogenesis may lead to improvements in diagnosis, treatment and prevention. Identification of novel biomarkers for cancer diagnosis and novel targets for treatment is a major goal [1]. Genes encoding transmembrane/secr

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family of genes belonging to the calcium-dependent lectin (C-type lectin) gene superfamily\(^{[3-6]}\). Reg represents a group of small secretory proteins that are essential for cell regeneration and proliferation and form an immune system\(^{[7,8]}\). Reg plays a wide range of roles in human physiology as well as in disease\(^{[2-4]}\) (Table 1). Among the human Reg family, special attention has been paid to Regulating islet-derived type IV (RegIV), the most recently discovered member. RegIV was originally isolated from a cDNA library of ulcerative colitis tissues by Hartupee et al\(^{[9]}\). It is expressed not only in various normal tissues such as stomach, colon, small intestine and pancreas\(^{[16,26]}\), but also in various malignant diseases, including colorectal\(^{[9]}\), gastric\(^{[10]}\), pancreatic\(^{[20]}\) and gallbladder cancer\(^{[21]}\). The biological functions of RegIV in cancers are not fully understood; however, several possible functions have been proposed.

**MOLECULAR CHARACTERISTICS OF REGIV AND OTHER REG GENES**

Using sequence analysis, Hartupee et al\(^{[9]}\) mapped the Reg IV gene to chromosome 1 and determined that the RegIV gene contains 6 exons, the structure of which is preserved among members of the Reg gene family. Zhang et al\(^{[22]}\) also showed that most members of the Reg family have similar organization with respect to exon number and chromosome location.

Previous studies have revealed that the Reg family shares strong similarities with C-type lectin\(^{[23]}\), which is distinguished from other lectins by sharing a calcium-dependent carbohydrate recognition domain (CRD). This domain of lectin might account for the complex events induced by RegIV and other Reg genes\(^{[23-25]}\). Unlike Reg IV, however, other Reg genes are present at low or undetectable levels in most tumors\(^{[21]}\). Analysis of the unique structural similarities and differences between RegIV and other members of this gene family are expected to provide a basis for investigations of structure-function relations in this gene family.

As for binding of RegIV to other molecules or putative receptors, detailed interactions have not been elucidated. Ho et al\(^{[26]}\) recently provided evidence that human RegIV binds to polysaccharides and mannan in the absence of calcium, unlike other C-type lectins. Utilizing nuclear magnetic resonance to elucidate the structural basis for carbohydrate recognition of RegIV, they found that RegIV has two calcium-independent mannan-binding sites serving as CRDs, suggesting a potential role in specific carbohydrate recognition\(^{[28]}\). These findings might provide clues to understanding the sugar-binding role of RegIV proteins, as well as molecular interactions with currently unknown receptors.

**REGIV OVEREXPRESSION IN GASTROENTEROLOGICAL CANCER**

Although various normal tissues express RegIV, expression levels are much lower in normal tissues than in cancerous tissues\(^{[27]}\). Violette et al\(^{[32]}\) showed that the RegIV is more strongly expressed in colorectal tumors (particularly in mucinous carcinomas) and normal small intestine than in normal colorectal tissue. They also demonstrated that RegIV-positive tumor cells display different phenotypes: mucus-secreting, enterocyte-like, or undifferentiated.

Several studies have demonstrated overexpression of RegIV in gastric cancer. Oue et al\(^{[29]}\) reported that RegIV expression was significantly higher in gastric carcinoma than in normal tissue on quantitative real-time polymerase chain reaction (PCR). Moreover, RegIV expression was associated with both intestinal mucin phenotype and neuroendocrine differentiation.

In pancreatic ductal adenocarcinoma\(^{[28]}\) as well as in gallbladder carcinoma\(^{[23]}\) amplification of RegIV in normal tissues was not apparent on quantitative real-time PCR. In contrast, high levels of RegIV were found in cancer tissues. These data suggest that overexpression of RegIV is associated with generating and maintaining cancer tissue.

**IMPORTANT ROLES OF REGIV IN CANCER TISSUES**

**Overexpression of RegIV is an early event in carcinogenesis**

Carcinogenesis is a multistep process involving somatic mutations or epigenetic changes affecting tumor suppressor genes and oncogenes. Several studies have indicated that RegIV may participate in early carcinogenesis in certain cancers.

Most colorectal carcinomas are thought to develop through the “adenoma-to carcinoma sequence” model\(^{[28]}\), in which adenomas are recognized as precursor lesions of the vast majority of colorectal cancers. Elucidation of the adenoma-carcinoma sequence along with its corresponding molecular genetic alterations will significantly enhance our understanding of the pathogenesis of colorectal carcinoma. However, since research on genetic mutations of adenomas is scant, the mechanism of the adenoma-adenocarcinoma sequence remains elusive.

Zhang et al\(^{[24]}\) generated a large collection of candidate, differentially expressed genes in primary colorectal adenomas and found that RegIV was one of the differentially expressed genes in colorectal adenoma and adenocarcinoma. They proposed that overexpression of RegIV may be an early event in colorectal carcinogenesis.

In gastric cancer, one of the important precancerous changes is intestinal metaplasia caused by chronic inflammation\(^{[29]}\). Oue et al\(^{[27]}\) performed serial analysis of gene expression of gastric carcinoma and identified several genes potentially involved in invasion, metastasis and carcinogenesis, and reported that RegIV is a candidate gene for cancer-specific expression. They also performed immunohistochemical analysis of RegIV in gastric tissues and showed that RegIV protein was immunohistochemically expressed in the goblet cells of intestinal metaplasia of the stomach and gastric carcinoma, suggesting an association of RegIV protein with intestinal differentiation of the
stomach and the pathogenesis of intestinal-type gastric carcinoma[61].

Gallbladder carcinoma is also thought to arise from epithelial dysplasia, and dysplasia appears to arise from metaplasia[45]. Tamura et al[35] reported that RegIV participates in gallbladder carcinogenesis via intestinal metaplasia because RegIV expression was found not only in the cancer cells but also in the intestinal metaplastic epithelium of patients with adenomyomatosis. In contrast, RegIV expression was never apparent in the normal epithelium of the gallbladder.

Intraduct papillary mucinous neoplasms (IPMN) of the pancreas show a wide spectrum of histological differentiation from hyperplasia and adenoma, and the existence of an adenoma-carcinoma sequence has been documented[31–33]. Aday et al[34,36] suggested that the intestinal-type IPMN to colloid carcinoma sequence is a distinct pathway of carcinogenesis involving intestinal-related genes caudal-type homeobox transcription factor 2 (CDX2) and Mucin 2 intestinal (MUC2). They described this pathway as the “intestinal” pathway of carcinogenesis. CDX2 is a transcriptional factor that is important for the maintenance of intestinal identity[37], and MUC2 is a major mucin detected in intestinal epithelium[38]. Nakata et al[39] analyzed RegIV and CDX2 expressions in patients with IPMNs using immunohistochemical staining and microdissection-based quantitative real-time reverse transcription PCR. The positive rates of both RegIV protein and mRNA expression were significantly higher in intestinal-type IPMN than in the other types of IPMN. A significant correlation between RegIV and CDX2 mRNA levels was also demonstrated. They concluded that RegIV plays an important role in differentiation of the “intestinal” pathway of IPMN and may be regulated by CDX2.

Carcinogenesis is a complex, multistep process involving somatic mutations or epigenetic changes affecting tumor suppressor genes and oncogenes[30,60]. RegIV may contribute a part of these processes, however further investigation is essential for comprehensive elucidation.

RegIV as an antiapoptotic factor

Advanced malignancies are often associated with poor responses to chemotherapy or radiation. Additional generic alterations in tumorigenesis create a permissive environment for clonal expansion of cells that are resistant to apoptosis[61]. So far, considerable attention has been given to the B-cell lymphoma 2 (Bcl-2) family of genes as possible regulators of intrinsic tumor resistance to therapy[42]. Repressors of programmed cell death, such as Bcl-2 and B-cell lymphoma-extra large (Bcl-xl), are known to decrease radiation- and chemotherapy-induced apoptotic cell death in cell cultures[43] via the Akt signaling pathway, which is an important determinant of the response to anticancer therapy[44]; overexpression of these genes suggests a poor prognosis in colon cancer[45]. However, key cellular factors that regulate expression of anti-apoptotic genes in tumors are not fully clarified. Defining dominant pathways responsible for regulating apoptosis could broaden current strategies for therapeutic intervention.

Bishunpuri et al[46] investigated possible roles of RegIV in colon cancer cells, using an in vitro radiation-survival colony assay. Colon cancer cells were cultured with or without recombinant human RegIV (rhR4) and exposed to 4 Gy of irradiation. After irradiation, colony counts increased significantly in rhR4-treated cell lines, but decreased in untreated cells. In the absence of irradiation, rhR4 treatment did not alter the numbers of colonies in treated cells. These data indicate that RegIV promotes tumor cell survival following a potent apoptotic stimulus. Furthermore, to establish a causative association between RegIV and the anti-apoptotic genes Bcl-2 and Bcl-xL, rhR4 was added to cultures of colon carcinoma cell lines, and Bcl-2 and Bcl-xL mRNA expression levels were analyzed. Both Bcl-2 and Bcl-xL expression levels increased significantly after rhR4-treatment in colon cancer cell lines, indicating that exogenous RegIV regulates expression of the Bcl-2 and Bcl-xL.

Mitani et al[47] also transfected gastric cancer cells with vector expressing RegIV to investigate the biologic significance of RegIV. To evaluate the effects of RegIV on the response to 5-fluorouracil (5-FU) treatment, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays were performed. Overexpression of RegIV in gastric cancer cells was confirmed to significantly inhibit 5-FU-induced apoptosis compared with cells transfected with empty vector on both MTT assay and measurement of DNA fragments. They also examined the expression of dihydropyrimidin dehydrogenase (DPD), because DPD has important roles in the pharmacokinetics and toxicity of 5-FU[47,48]. Induction of DPD expression was demonstrated in cells overexpressing RegIV compared with a negative control on Western blotting. They next examined the relevance of RegIV expression to the response of gastric cancer to low-dose 5-FU and cisplatin in patients with...
Regulatory IV (Reg IV) has received interest in gastroenterological cancers due to its proinvasive and anti-apoptotic properties. In colorectal cancer, Reg IV expression has been associated with poor prognosis and is related to metastatic progression. For gastric cancer, it has been linked to disease recurrence and poor survival rates. The anti-apoptotic effect of Reg IV contributes to lymph node metastasis and resistance to chemoradiotherapy.

**Table 2: Articles reporting regenerating islet-derived type IV as anti-apoptotic factor**

| Author          | Year | Type of cancer | Result                                                                 |
|-----------------|------|----------------|------------------------------------------------------------------------|
| Bishnupuri et al. | 2006 | Colorectal     | Reg IV induces cell survival against radiation, and regulates Bcl-2 and Bcl-xL. |
| Mitani et al.   | 2007 | Gastric        | Reg IV inhibited 5-fluorouracil-induced apoptosis, and induced DPD expression |
| Eguchi et al.   | 2009 | Pancreatic     | Reg IV decreased the sensitivity to radiation and gemcitabine           |

Reg IV: Reporting regenerating islet-derived type IV; Bcl-2: B-cell lymphoma; Bcl-xL: B-cell lymphoma-extra large; DPD: Dihydropyrimidine dehydrogenase.

Reg IV has been shown to induce cell survival against radiation and regulate Bcl-2 and Bcl-xL. In colorectal cancer, Oue et al. demonstrated that lymph node metastasis was positive in 69.2% of the Reg IV-positive group, but only 30.4% of Reg IV-negative group, suggesting that Reg IV contributes to lymph node metastasis in colorectal cancer. A relation between overexpression of Reg IV and liver metastasis has also been demonstrated in patients with colorectal cancer. One study of 30 patients with colorectal cancer showed that metastatic recurrence in the liver during follow-up was more frequently associated with the presence than the absence of Reg IV expression.

**Reg IV as a proinvasive factor**

Rafa et al. investigated the potential function of Reg IV as a proinvasive factor in colorectal cancer cells. Colon cancer cells secreting Reg IV or not were used to analyze the autocrine and paracrine effects of Reg IV. They evaluated the invasive properties of cancer cells by performing collagen type I invasion assays and calculating invasion index, which is useful for judging the invasion ability. They demonstrated that cell lines which secreted Reg IV were spontaneously invasive, whereas cells which did not secrete Reg IV were non-invasive compared with positive control cells. They also added the Reg IV protein to non-Reg IV-secreting cell lines, and confirmed a dose-dependent increase in the invasive index. Addition of an anti-Reg IV antibody to assays with the invasive cell lines significantly limited their invasive properties. These results suggest that Reg IV promotes in vitro invasion of colon cancer cell lines in both an autocrine and a paracrine manner.

**Reg IV EXPRESSION AND CLINICOPATHOLOGICAL FEATURES**

Several studies have contrasted clinicopathological features with expression levels of Reg IV. Among 36 patients with colorectal cancer, Oue et al. demonstrated that lymph node metastasis was positive in 69.2% of the Reg IV-positive group, but only 30.4% of Reg IV-negative group, suggesting that Reg IV contributes to lymph node metastasis in colorectal cancer. A relation between overexpression of Reg IV and liver metastasis has also been demonstrated in patients with colorectal cancer. One study of 30 patients with colorectal cancer showed that metastatic recurrence in the liver during follow-up was more frequently associated with the presence than the absence of Reg IV expression.

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**Reg IV as a prognostic factor**

Reg IV is a prognostic factor in colorectal cancer. In gastric cancer, positive Reg IV expression has been associated with poor differentiation of tumors, although there was no clear correlation between Reg IV expression and tumor depth or lymph node metastasis. The results of an in vitro study by Kuniyasu et al. suggest that Reg IV might accelerate peritoneal metastasis in gastric cancer.

These studies generally discussed the unfavorable impact of Reg IV expression on clinicopathological features. Cell proliferation and anti-apoptotic effect induced by Reg IV may accelerate progression of cancer.
mor size, liver metastasis, and a higher level of RegIV gene expression \((P = 0.029)\) were significant independent predictors of overall survival in colorectal cancer\(^{[55]}\). Miyagawa et al\(^{[53]}\) estimated RegIV mRNA levels in the peritoneal washes of 95 patients with gastric cancer by real-time reverse transcription-PCR. The RegIV mRNA level was correlated with the extent of wall penetration and peritoneal metastases. They also found that the outcomes of RegIV-positive patients were significantly worse than those of RegIV-negative patients. Multivariate analysis suggested that RegIV is an independent prognostic factor\(^{[56]}\).

Tao et al\(^{[58]}\) measured RegIV mRNA levels by immunohistochemical staining of tissue, and enzyme-linked immunosorbent assay of serum. Their results confirmed that the mean survival time was significantly shorter in patients with RegIV-positive gastric cancer than in those with RegIV-negative gastric cancer \((P = 0.013)\). In gallbladder cancer, Tamura et al\(^{[21]}\) reported that high expression of RegIV correlated with a well-differentiated phenotype accompanied by better outcomes, where lower expression correlated with a poorly differentiated phenotype accompanied by worse survival, suggesting that loss of RegIV expression might be associated with more malignant characteristics.

According to these data, RegIV expression is generally associated with poor outcomes in colorectal cancer and gastric cancer. Further studies will hopefully clarify differences in clinical outcomes according to the type of cancer.

### RegIV as a diagnostic biomarker

Serum biomarkers for the detection of cancer are needed in order to find a larger number of candidates for suspected cancer. Several studies have assessed the feasibility of using RegIV as a serum diagnostic marker (Table 3). Oue et al\(^{[51]}\) measured serum RegIV levels in patients with colorectal cancer by enzyme-linked immunosorbent assay to investigate the diagnostic potential of RegIV. Increased preoperative levels of RegIV were found in a low numbers of serum samples from patients with stage 0–III colorectal cancer, indicating that serum RegIV is unsuitable for the detection of early colorectal cancer. In contrast, in patients with stage IV disease, the serum RegIV concentration was significantly higher in the presence than in the absence of liver metastasis, suggesting that RegIV is a good marker for metastatic recurrence in the liver after resection of colorectal cancer.

Mitani et al\(^{[49]}\) demonstrated that the serum RegIV concentration in presurgical patients with gastric cancer was significantly higher than that in healthy individuals \((1.96 \pm 0.17 \mu g/L \text{ vs } 0.52 \pm 0.05 \mu g/L, P < 0.001)\). The diagnostic sensitivity of serum RegIV \((36.1\%)\) was superior to that of serum carcinoembryonic antigen \((CEA, 11.5\%)\) or carbohydrate antigen 19-9 \((CA19-9, 13.1\%)\).

Kobayashi et al\(^{[50]}\) also evaluated the usefulness of serum RegIV levels as a diagnostic marker for gastric cancer. They collected pretreatment serum samples from patients with gastric cancer and healthy control subjects without cancer. RegIV levels were significantly higher in patients with early gastric cancer \((\text{median } 8.42 \mu g/L)\) than in the control subjects \((\text{median } 5.01 \mu g/L)\) \((P < 0.001)\). RegIV levels were also higher in patients with advanced gastric cancer \((\text{median } 13.12 \mu g/L)\) than in those with early gastric cancer \((P < 0.02)\). The sensitivity for gastric cancer was 73.0\%, the specificity was 70.8\%, and the accuracy was 71.8\%, which is superior to the respective values for CEA and CA19-9. Serum RegIV levels were thus suggested to be potentially useful as a screening marker for gastric cancer, including early disease.

In pancreatic cancer, Takehara et al\(^{[20]}\) detected significantly elevated serum RegIV levels, measured with the use of an enzyme-linked immunosorbent assay system, in patients with early-stage pancreatic ductal adenocarcinomas. Their findings suggested that RegIV may become a new serological marker of pancreatic ductal adenocarcinoma.

Serum RegIV level can be a useful indicator to distinguish between patients with cancer and healthy subjects. RegIV has the potential to be used as a screening serum marker for certain cancers, including cancers in the early stages.

### SIGNALING PATHWAY OF REGIV, AND REGIV-TARGETED THERAPY

Monoclonal antibody therapy has become an important option for the management of gastroenterological cancer. Bevacizumab, a humanized monoclonal antibody to vascular endothelial growth factor, is currently approved in combination with intravenous 5-FU-based regimens for first-line treatment of metastatic colorectal cancer. Besides the anti-angiogenesis factor antibody, antibodies against circulating ligands, such as hepatocyte growth factor\(^{[50]}\) and interleukin-6\(^{[51]}\), are under review as anticancer drugs.

The signaling pathway activated by RegIV is poorly understood; however, Bishunpuri et al\(^{[52]}\) recently demonstrat-
Recent studies revealed that the serum RegIV gene as related to gastroenterological cancer, focusing on its role in cancer tissue and its impact on clinical outcomes. RegIV is generally upregulated in gastroenterological cancers, including those of the stomach, colorectum, and pancreas, as well as in benign diseases such as ulcerative colitis. Available evidence suggests that the basic biological effects of RegIV seem to be induction of cellular proliferation, invasion and inhibition of apoptosis, resulting in relatively worse clinicopathological features, or worse survival in patients with high-RegIV expression than those without.

Recent studies revealed that the serum RegIV level can be a novel biomarker to detect patients with colorectal, gastric, and pancreatic cancer. These studies suggested RegIV has the potential to be used as a screening serum.
marker. In addition, our multivariate analysis suggested that overexpression of the RegIV gene is a useful prognostic biomarker in patients with colorectal cancer, which corresponds to other reports.

The signaling pathway activated by RegIV is not fully understood. However, recent studies demonstrated that RegIV is a potent activator of the EGFR/Akt signaling cascade, which is associated with cell survival and proliferation. Further investigation of RegIV, particularly its cell surface receptors and signaling pathways, will further our understanding of the basic mechanisms of this gene. Strategies designed to reduce endogenous RegIV expression or to block downstream signaling warrant additional investigations to delineate their potential roles in the prevention or treatment of established gastrointestinal adenocarcinomas.

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| Author | Year | Method/type of cancer | Result |
|--------|------|-----------------------|--------|
| Bishumpuri et al | 2006 | Transfection of rhR4/colorectal | Increase in pEGFR and pAkt |
| Takehara et al | 2006 | Transfection of rhR4 and siRNA/pancreatic | Significant reduction in viable cells and increase in pAkt |
| Legoff et al | 2009 | RegIV antibody injection/pancreatic | Significant reductions in Akt, Bcl-2 and Bcl-xl |

rhR4: Recombinant human regenerating islet-derived type IV; RegIV: Human regenerating islet-derived type IV; siRNA: Small interfering RNA; pEGFR: Phosphorylated epidermal growth factor receptor; pAkt: Phosphorylated protein kinase B; Akt: Protein kinase B; Bcl-2: B-cell lymphoma 2; Bcl-xl: B-cell lymphoma-extra large.
Numerous studies have elucidated the molecular mechanisms underlying the development and progression of colorectal cancer. The expression of mucin genes, particularly MUC1 and MUC2, has been shown to play a crucial role in the formation of invasive carcinomas. Mucins are glycoproteins that act as protective barriers and modulate cell-cell and cell-matrix interactions. In colorectal cancer, overexpression of MUC1 and MUC2 has been associated with more aggressive tumor behavior and worse clinical outcomes.

Dysregulation of the Wnt signaling pathway has been implicated in the development of colorectal neoplasms. The Wnt pathway is a complex intracellular signaling network that regulates cell proliferation, differentiation, and survival. In colorectal cancer, mutations in the Wnt pathway components have been found to result in aberrant activation of this signaling cascade, leading to the persistence of precursor lesions and the initiation of malignant transformation.

The hedgehog (HH) signaling pathway, another key regulator of cell proliferation and differentiation, is also frequently altered in colorectal cancer. The activation of HH signaling has been linked to increased cell proliferation, invasion, and metastasis in colorectal cancer cells. The inhibition of HH signaling has shown promise as a potential therapeutic strategy to prevent the growth and spread of colorectal cancer.

In the preclinical setting, the targeting of key signaling pathways, such as the Wnt and HH pathways, has been evaluated in preclinical models. The use of small-molecule inhibitors and targeted antibodies has demonstrated efficacy in blocking the pro-tumorigenic effects of these pathways. Preclinical studies have shown that targeting the Wnt and HH pathways can lead to a reduction in tumor burden and improved survival in colorectal cancer models.

In the clinic, the treatment strategies for colorectal cancer are guided by the specific molecular alterations present in each patient. The prognosis of colorectal cancer is significantly influenced by the presence of mutations in key oncogenes and tumor suppressor genes. Patients with specific genetic profiles may benefit from targeted therapies that exploit these alterations.

In conclusion, the molecular mechanisms underlying colorectal cancer development and progression are multifaceted and involve complex signaling pathways. Advances in our understanding of these pathways have paved the way for the development of targeted therapies that promise to improve outcomes for patients with colorectal cancer.
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S-Editor Cheng JX  L-Editor Cant MR  E-Editor Xiong L