The role of Reg IV in colorectal cancer, as a potential therapeutic target

Yiwang Hu[1], Chi Pan[1], Jiyi Hu[2], Suzhan Zhang[1]

[1]Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education), School of Medicine, Zhejiang University, Hangzhou, PR China
[2]Medical Oncology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, PR China

Regenerating islet-derived family, member 4 (Reg IV), a member of the Reg gene family, has been reported to be overexpressed in gastrointestinal tract cancers. Reg IV overexpression in tumor cells has been associated with carcinogenesis, tissue regeneration, proliferation and resistance to apoptosis. Reg IV activates the epidermal growth factor receptor (EGFR) signaling pathway in colon cancer and increases expression of B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xl), which are associated with the inhibition of apoptosis, results in mitogenic signaling in colon cancer cells, increase cell proliferation, metastasis and decreased apoptosis. Reg IV treatment inhibits 5-fluorouracil induced apoptosis, at least two mechanisms are involved in inhibition of apoptosis by Reg IV, including Bcl-2 and dihydropyrimidine dehydrogenase (DPD). These studies may lead to novel therapeutic strategies for cancers expressing Reg IV. Recently, one proteoglycan was confirmed to disrupt this signaling pathway to perform antitumor effect. This review summaries current knowledge of the expression and roles of Reg IV in human colorectal cancer, describes the possible signaling pathway which Reg IV activates, and discusses the relevance of Reg IV as a potential therapeutic target for cancer treatment.

Key words: Reg IV, colorectal cancer, Cdx2, EGFR/Akt/AP-1 pathway, targeted therapy.

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Introduction

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 [1]. Despite improvements in cancer diagnosis and therapy, many patients are still diagnosed at the late stages of the disease, and the disease often recurs even after curative surgery. Multiple genetic alterations lead to carcinogenesis. Research on the candidate differentially expressed genes may help us to find biological markers for the evaluation of cancer diagnosis and offer novel molecular targets for anticancer therapy.

Over the past three decades, considerable attention has focused on the Reg (regenerating) gene family, which belongs to the calcium-dependent lectin superfamily, encoding a group of small multifunctional secretory proteins [2]. Reg family proteins are primarily involved in cell proliferation and differentiation, inflammation, diabetes, and carcinogenesis [3]. Regenerating islet-derived family member 4 (Reg IV), the most recently discovered member of the Reg gene family, is thought to be a candidate gene for cancer-specific expression [4]. In this review, we summarize current understanding of Reg IV, and discuss the relevance of Reg IV as a potential therapeutic target in the treatment of colorectal cancer.

Expression and function of Reg IV in gastrointestinal tract tumors

Reg IV is expressed in a large variety of normal tissues in humans, such as stomach, small intestine, colon and pancreas [2, 5]. The expression of Reg IV is abundantly enhanced in colorectal adenocarcinoma and adenoma [6, 7], and gastric cancer [5, 8], whereas Reg IV expression is not detected in lung or breast cancers [5]. Therefore, Reg IV may be a good marker for gastrointestinal tumors. Previous studies have suggested that Reg IV may take part in early carcinogenesis in certain cancers. Many colorectal cancers develop through the 'adenoma to carcinoma sequence' model [9], in which adenomas are recognized as precursor lesions of the vast majority of colorectal cancers. Zhang *et al*. found that Reg IV was expressed in both colorectal adenoma and adenocarcinoma [7]. This result constituted further evidence that overexpression of Reg IV may be an early event in the colorectal adenoma-carcinoma sequence and carcinogenesis, and its detection may be useful in the early diagnosis of colorectal adenoma formation [10].

Whether Reg IV is associated with tumor clinicopathological features is unknown yet. Violette *et al*. reported that there was no significant relationship between Reg IV and the TNM state of the tumors or their localization [6]. But Yamagishi *et al*. suggested that Reg IV staining was observed more fre-

quently in stage III/IV cases than in stage I/II cases, and patients with Reg IV positive gastric cancer tended to show a poor outcome, although not to a statistically significant degree [11]. In contrast to gastric carcinoma, expression of Reg IV by colorectal carcinoma is associated with lymph node metastasis. Numata *et al.* showed that high expression levels of Reg IV were correlated with well-differentiated histological type, deeper invasion, presence of lymphatic invasion, presence of liver metastasis, and advanced stage (stage IV) [12]. Oue *et al.* reported that Reg IV expression was associated with delayed liver metastasis of colorectal cancer [13], and inhibition of apoptosis by Reg IV may participate in liver metastasis. It has also been reported that Reg IV induces the expression of matrix metalloproteinase-7 (MMP-7) [14], which promotes liver metastases [15]. Overexpression of Reg IV may contribute to liver metastasis through induction of MMP-7.

Transcription of the Reg IV gene may be regulated by Cdx2

The caudal homeobox 2 gene (Cdx2) is reported to be involved in colorectal carcinogenesis [16] as well as the status of its differentiation [17], and is known to be a tumor suppressor [18]. The prognosis of patients with negative Cdx2 expression is significantly poorer than patients with positive expression. To date, little is known about the relationship between Reg IV and Cdx2 expression. It is reported that Mucin-2 (MUC2) and Reg IV were both positive in goblet cell-like vesicles in tumor cells, and both mucin-like staining and perinuclear Reg IV staining are observed more frequently in MUC2 positive cases than in MUC2 negative cases [5]. Yamamoto *et al.* [19] and Werling *et al.* [20] found that Cdx2 interacts with the MUC2 promoter and activates MUC2 transcription. What is more, Naito *et al.* showed that Cdx2 protein directly regulates Reg IV expression [21]. Taken together, we suppose that Cdx2 may regulate transcription of the Reg IV gene.

Reg IV activates the EGFR/Akt/AP-1 signaling pathway

Although the biological function of Reg IV is poorly understood, Reg IV is expressed in almost all epidermal growth factor receptor (EGFR) positive gastric cancers [8] and colorectal cancers [14, 22]. Reg IV may activate EGFR and may contribute to cancer cell growth. Bishnupuri *et al.* found that Reg IV is a potent activator of the EGFR/Akt/activator protein-1 (AP-1) signaling pathway in colon cancer cells and increases expression of B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xl), which are proteins associated with the inhibition of apoptosis [14, 23, 24]. Activation of the EGFR signaling pathway resulted in mitogenic signaling in colon cancer cells, increased cell proliferation, angiogenesis, and metastasis, and reduced apoptosis [25]. Epidermal growth factor receptor activation indicates poor prognosis, and increases invasiveness of carcinomas and resistance to apoptotic cell death. Reg IV treatment induces tyrosine phosphorylation of the EGFR and inhibits 5-fluorouracil-induced apoptosis in gastric cancer [8].

Reg IV as a therapeutic target in colorectal cancer

It is reported that advanced colorectal adenocarcinoma is generally poorly responsive to chemotherapy and radiation [26, 27], and patients with Reg IV positive gastric cancer tend to show a poor outcome [28]. As demonstrated in other systems [13, 29], there are diverse mechanisms of resistance to drug toxicity. 5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic agents for breast cancer, colorectal cancer, and gastric cancer [30]. Unfortunately, some patients show a poor response. High Reg IV expression is thought to be associated with 5-FU resistance in colon cancer cell lines [6].

Possible mechanisms of Reg IV inhibition of apoptosis

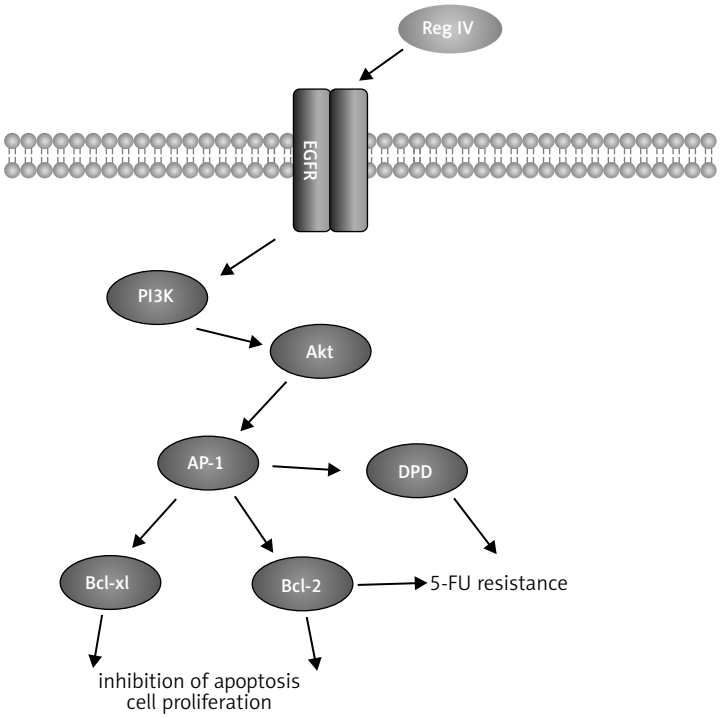
It has been reported that recombinant Reg IV increases expression of Bcl-2 [14]. B-cell lymphoma-2 is an antiapoptotic protein located on mitochondria and is expressed at high levels in some tumor cells and tissues [24, 31]. In the mitochondrial pathway, antiapoptotic Bcl-2 family proteins prevent mitochondrial membrane permeabilization and thereby inhibit changes in the mitochondrial membrane potential and cytochrome c release [31]. Mitani *et al.* suggested that overexpression of Reg IV may suppress 5-FU-induced apoptosis by inhibiting the mitochondrial apoptotic pathway [8].

It has been reported that AP-1 induces expression of dihydropyrimidine dehydrogenase (DPD) [32], which is an initial and rate-limiting enzyme in 5-FU catabolism, and has significance for the pharmacokinetics and toxicity of 5-FU. Overexpression of DPD in tumor cell lines is associated with resistance to 5-FU [33]. Degradation of 5-FU by induction of DPD expression may also inhibit 5-FU-induced apoptosis. Also, Mitani *et al.* confirmed that Reg IV inhibits apoptosis by activating the EGFR/Akt/AP-1/DPD signaling pathway, inhibiting 5-FU.

In conclusion, Reg IV can confer resistance to 5-FU-induced apoptosis in colorectal cancer and gastric cancer. At least two mechanisms are involved in inhibition of apoptosis by Reg IV, including Bcl-2 and DPD. Bishnupuri *et al.* reported that Reg IV is a potent activator of the EGFR/Akt/AP-1 signaling pathway in human colon cancer cell lines [14]. Interestingly, Nanakin *et al.* found that EGF and TGF-α enhanced Reg IV gene expression by the extracellular signal-regulated kinase (ERK) signaling pathway in the SW403 cells line [22], and high phospho-ERK expression is associated with a lower survival rate of colorectal cancer [34]. These studies suggest that a positive expression feedback loop between EGF and Reg IV exists in the signaling pathways. So disruption of Reg IV/EGFR signaling may have utility as a novel therapeutic intervention for human gastrointestinal cancer.

The proteoglycan 'P1' disrupts the Reg IV/EGFR/Akt/AP-1 signaling pathway

Li *et al.* suggested that a proteoglycan named 'P1' which is from *Phellinus linteus* (PL) could disrupt the Reg IV/EGFR/Akt/AP-1 signaling pathway [35]. They found that



PI3K – phosphoinositide 3-kinase; AP-1 – activator protein-1; DPD – dihydropyrimidine dehydrogenase; Bcl-2 – B-cell lymphoma-2; Bcl-xl – B-cell lymphoma-extra large

Fig. 1. Reg IV activates the EGFR/Akt/AP-1 signaling pathway. Regenerating islet-derived type IV (Reg IV) could activate the epidermal growth factor receptor (EGFR) signaling pathway in colon cancer cells and increases expression of Bcl-xl and Bcl-2, resulting in cell proliferation and inhibition of apoptosis. B-cell lymphoma-2 prevents mitochondrial membrane permeabilization and leads to 5-FU resistance. Dihydropyrimidine dehydrogenase is an initial and rate-limiting enzyme in 5-FU catabolism, which can be induced by AP-1

treatment of colonic adenocarcinoma cells with P1 resulted in significant dose-dependent inhibition in cell numbers and cell mitosis. P1 had the capacity to downregulate the expression of Reg IV and EGFR. Therefore, the proteoglycan P1 was considered to block the EGFR signaling pathway and induce Reg IV downregulation. Also, a study *in vivo* confirmed this hypothesis. P1 has a direct antitumor effect through inducing apoptosis and inhibiting the karyokinesis of HT-29 cells. The results are consistent with the previous report by Li *et al.* [36]. Therefore we expect that it will be possible to use P1 as an adjuvant chemotherapeutic and chemopreventive agent. Numerous proteins in the Reg IV/EGFR/Akt/AP-1 signaling pathway are potential therapeutic targets for colorectal cancer treatment awaiting discovery.

Conclusions and future perspective

This review focuses on the expression and roles of Reg IV in gastrointestinal tract cancers. Reg IV is generally upregulated in gastrointestinal tract cancers. Available evidence suggests that Reg IV seems to have functions, including promoting tissue regeneration, proliferation, and resistance to apoptosis, resulting in relatively worse clinicopathological features, or worse survival in patients with high Reg IV expression than those without. Reg IV is expressed in colorectal adenoma and intestinal metaplasia of the stomach, and is considered to be a good potential marker for gastrointestinal tumors. Reg IV activates the EGFR/Akt/AP-1 signaling pathway in colon cancer cells, increases cell proliferation, angiogenesis, and metastasis, and reduces apoptosis. Reducing endogenous Reg IV expression or blocking downstream signaling may be a feasible therapeutic strategy. Further investigations are still needed to confirm these observations and find more anti-cancer agents targeting the Reg IV/EGFR/Akt/AP-1 signaling pathway.

The authors declare no conflict of interest.

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Address for correspondence

Suzhan Zhang
Cancer Institute (Key Laboratory
of Cancer Prevention and Intervention,
China National Ministry of Education)
School of Medicine, Zhejiang University
Hangzhou, 310000, People's Republic of China
e-mail: Zhangscy07@gmail.com

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