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• REVIEW •

# Reg gene family and human diseases

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## Abstract

Regenerating gene (Reg or REG) family, within the superfamily of C-type lectin, is mainly involved in the liver, pancreatic, gastric and intestinal cell proliferation or differentiation. Considerable attention has focused on Reg family and its structurally related molecules. Over the last 15 years, 17 members of the Reg family have been cloned and sequenced. They have been considered as members of a conserved protein family sharing structural and some functional properties being involved in injury, inflammation, diabetes and carcinogenesis. We previously identified Reg IV as a strong candidate for a gene that was highly expressed in colorectal adenoma when compared to normal mucosa based on suppression subtractive hybridization (SSH), reverse Northern blot, semi-quantitative reverse transcriptase PCR (RT-PCR) and Northern blot. *In situ* hybridization results further support that overexpression of Reg IV may be an early event in colorectal carcinogenesis. We suggest that detection of Reg IV overexpression might be useful in the early diagnosis of carcinomatous transformation of adenoma. This review summarizes the roles of Reg family in diseases in the literature as well as our recent results of Reg IV in colorectal cancer. The biological properties of Reg family and its possible roles in human diseases are discussed. We particularly focus on the roles of Reg family as sensitive reactants of tissue injury, prognostic indicators of tumor survival and early biomarkers of carcinogenesis. In addition to our current understanding of Reg gene functions, we postulate that there might be relationships between Reg family and microsatellite instability, apoptosis and cancer with a poor prognosis. Investigation of the correlation between tumor Reg expression and survival rate, and analysis of the Reg gene status in human maliganancies, are required to elucidate the biologic consequences of Reg gene expression, the implications for Reg gene regulation of cell growth, tumorigenesis, and the progression of cancer. It needs to be further attested whether Reg gene family is applicable in early detection of cancer and whether Reg and Reg-related molecules can offer novel molecular targets for anticancer therapeutics. This has implications with regard to prognosis, such as in monitoring cancer initiation, progression and recurrence, as well as the design of chemotherapeutic drugs.

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## INTRODUCTION

Reg and Reg-related genes constitute a family belonging to calcium dependent lectin (C-type lectin) gene superfamily[1-4]. It represents a group of small secretory proteins, which can function as acute phase reactants, lectins, antiapoptotic factors or growth factors for pancreatic β-cells, neural cells and epithelial cells in the digestive system[5,6]. They play a wide range of roles in researching mammal physiology and human diseases. Ever since Reg (regenerating gene) I was discovered, special attentions have been paid to the regeneration of pancreatic β-cells and administration of Reg I protein and/or activation of the Reg I gene to be used as a potential therapeutic approach for diabetes[7]. Successively, the potential role of Reg family in tumors especially in digestive tract has drawn more attention[8-14]. We here focus on the members of Reg family, their functions and possible mechanisms.

## REG FAMILY

### *Discovered members*

In 1984, Yamanoto *et al*. found that administration of nicotinamide accelerated the regeneration of pancreatic islets in partially pancreatectomized rats. Subsequently, Terazono *et al.* screened a rat regenerating islet-derived cDNA library and isolated a novel gene encoding a 165 amino acid protein with a 21 amino acid signal peptide[15,16], which was called Reg gene. It was not termed Reg I until 1997. They also cloned human Reg I cDNA encoding a 166 amino acid protein with a 22 amino acid signal peptide. Reg I has other synonyms such as PTP (pancreatic thread protein), PSP (pancreatic stone protein) and lithostathine[17]. Human Reg I gene is a single copy gene spanning 3.0 kb, and is composed of six exons and five introns. The gene mRNA was detected predominantly in the pancreas, and at lower levels in gastric mucosa and kidneys[18]. Later they isolated two genes, one of which was a mouse homologue to rat and human Reg gene, the other a novel type of Reg gene. The two genes were designated as Reg I and Reg II, respectively.

In 1999, Okamoto grouped the members of the family, Reg and Reg-related genes from human, rat and mouse, into three subclasses, types I, II, and III[19]. Stephanova *et al.* determined that the three rat PAP genes and the related Reg gene (REGL, regenerating islet-derived-like/ pancreatic stone protein-like/ pancreatic thread protein-like) were all located at 4q33-q34[20]. The mouse Reg family genes were mapped to a contiguous 75 kb region in chromosome 6, including Reg I, Reg II, Reg III alpha, Reg III beta, Reg III gamma, and Reg III delta[21]. Reg III delta was expressed predominantly in exocrine pancreas, whereas both Reg I and Reg II were expressed in hyperplastic islets and Reg III alpha, Reg III beta and Reg III gamma were expressed strongly in the intestinal tract and weakly in pancreas.

Although Reg IV (1q12-q21), identified by Hartupee *et*

*al*, has not been found in the same chromosome as other members of human Reg gene and Reg-related gene (2p12), it shares some common features with other members such as: sequence homology, tissue expression profiles, and exon-intron junction genomic organization[1]. Thus by 2001, four types of Reg gene family had been identified. Data of RT-PCR results in our laboratory were consistent with the hybridization result of Hartupee and colleagues, and Reg IV mRNAs level was higher in colon than in rectum. We compared the results with Reg I expression pattern. Kawanami *et al*. discovered that expression of Reg I mRNA was higher in the stomach than in any other region of the gastrointestinal tract[22], which also suggested that Reg mRNA was higher in proximate gastrointestinal tract.

**Table 1** Members of Reg family, length of amino acids and chromosome localization

| Superfamily memeber | Species | Length of amino aAcid | Chromosome localization |
|---|---|---|---|
| Reg I | Mouse Reg I | 165 | 6 |
| | Rat Reg | 165 | 4q33-q34 |
| | Human Reg / PSP / PTP | 166 | 2p12 |
| Reg II | Mouse Reg II | 173 | 6 |
| Reg III | Rat PAP | 175 | 4q33-q34 |
| | Rat peptide 23 | 175 | 4q33-q34 |
| | Human HIP | 175 | 2p12 |
| | Bovine PTP | 175 | |
| Reg IV | Human Reg IV | 158 | 1q12-q21 |

So far, 17 members have been identified in mammals across human, pig, mouse, bovine and rat species. Table 1 lists some most important members of Reg family[23,24]. Among the mammalian members of this family, there is only mouse Reg II in type II and human Reg IV in type IV. Hartupee *et al*. also reported that a mouse homologue of Reg IV was likely existed[1], but up to now, there are no reports and also no investigations on mouse Reg IV.

### *Structure and function*

Most members of Reg family have similar organization with respect to exon number and chromosome location. The most interesting characteristic is its common domain of lectin. Data have revealed a significant similarity of the sequences of Reg family with the C-type (Calcium-dependent) lectin superfamily. This domain of lectin could account for complex events such as human malignancy and other diseases[25,26].

Studies on Reg I protein receptor (Reg-R) revealed that regenerating protein might act not only as a regulator of gastric epithelial cell proliferation but also as a modifier of many other multiple physiologic functions[27,28]. Reg-R gene was isolated from a rat islet cDNA library[27] encoding a cell surface 919-amino acid protein. Its expression was detected mainly in chief cells and parietal cells of the deep layers and faintly in surface epithelial cells and mucous neck cells of the proliferating zone[29]. Reg I protein could induce β-cell proliferation via the Reg I receptor and ameliorate experimental diabetes[30].

Under physiological conditions, Reg I protein is not expressed in pancreatic β-cells, although Reg-R is expressed. In the regenerative process of pancreatic islets, Reg I gene expression is induced. Therefore, activation of Reg I gene is thought to be one of the important events in β-cell regeneration.

## REG FAMILY AND HUMAN DISEASES

### *Injury response and inflammation*

Regenerating gene family members are expressed in tissue injury. As tissue injury is concerned, pancreatitis is most frequently studied. Experimental induction of acute pancreatitis caused a coordinate increase both in PSP/reg (Reg I) and in PAP (Reg III). Since the regulation of this protein family was affected even under mild stress, they were defined as secretory stress proteins[31-33]. Reg levels are sensitive markers for pancreatic injury and early stage of the disease, which might be useful prognostic indicators for disease severity[34]. The expression level of PSP/Reg I protein varies with different degrees of injury. Mild to moderate injury to pancreatic tissue might stimulate the synthesis of PSP/reg-protein, whereas more severe injury tended to depress it[32,34].

There are other evidences supporting Reg' s roles in the healing of gastrointestinal mucosal lesions. Miyaura *et al*. measured Reg expression after implantation or resection of a solid insulinoma in rats and found that the diminution in pancreatic β-cell mass caused by subcutaneous implantation of insulinoma tissues was associated with reduced Reg I gene expression and increase of β-cell proliferation after resection of the tumor was preceded by return of Reg I gene expression toward normal[35]. In an injured state following indomethacin treatment, Reg I gene expression was sharply increased, accompanied by an overexpression of c-fos and healing of mucosal lesions[22]. In addition, Reg I mRNA was detected predominantly in the deepest mucosal layer. It was expressed almost exclusively in cells that were less than 11 μm in diameter, which suggested a role of Reg I in the healing.

This also may be one of reasons why Reg genes have been frequently screened as differentially expressed genes[8,10,36-38]. Shinozaki *et al*. isolated seven candidate genes that were presumed to be up-regulated in inflammatory colonic epithelia and Reg I was among them. Expression of Reg I alpha was confined to the crypt epithelia[36] and its selective expression in the crypt epithelia of inflammatory colonic mucosa might suggest its important regulatory functions.

Another interesting change was the length of Reg mRNA. The elongated mRNA of PAP II/Reg III was strongly induced in the early phase after acute pancreatitis. The elongated mRNA might affect the function of PAP II/Reg III protein because the elongated mRNA with long 3' untranslated regions (3' UTR) was involved in the translation efficiency and thus played an important role in the progression of pancreatitis[39].

### *Diabetes*

Islet cells originate from the epithelial cells of primitive pancreatic ducts during embryogenesis, and can regenerate in response to the loss of islet cells even in adult pancreas. The ability of islet cells to regenerate could increase the possibility, which could restore the impaired and decreased islets of diabetic patients[40]. On the other hand, aging may be associated with selective dysfunction of β-cells, which may involve the expression of Reg I gene. Reg I gene could play an important role in β-cell growth/regeneration[41,42] and its expression could parallel to islet physiology, thus Reg I gene may become one of the targets of genetic engineering for diabetic β-cells.

In early 1980s, Takasawa *et al.* proposed a unifying model for β-cell damage (the okamoto model). In 1984, they demonstrated Reg I protein could induce β-cell proliferation and ameliorate experimental diabetes. Later, they showed that combined addition of IL-6 and dexamethasone could induce Reg I gene expression in β-cells and that inhibitors could enhance the expression[43]. In 2002, they reported that PARP and its inhibitors had key roles in inducing β-cell regeneration, maintenance of insulin secretion, and prevention of β-cell death[28].

The expression of Reg is a defense mechanism of the exocrine pancreas that is conserved in evolution. Sanchez *et al*. demonstrated that pancreatic Reg I and Reg II genes were overexpressed in non-obese diabetic (NOD) mice during active diabetogenesis[44,45]. They suggested that overexpression of the Reg gene(s) might represent a defense of acinar cells against pancreatic aggression. Although some results were opposite to their hypothesis[46,47], they further confirmed their previous findings by conducting the same protocol as Fu did.

Studies on differentially expressed genes have added proof to reveal Reg' s potential application in treatment. As we know, genes overexpressed in pancreatic islets of patients with diabetes are potential candidates for novel disease-related autoantigens. Subtractive hybridization was used on islets from a patient who died at the onset of type I diabetes, and a type I diabetes-related cDNA encoding hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (HIP/PAP, Reg III) was identified[48]. In addition, treatment aimed at abrogation of autoimmunity combined with expansion of β-cell mass has become a potential therapeutic approach for the treatment of insulin-dependent diabetes[49]. Therefore, diabetes might be ameliorated with Reg protein treatment.

### *Tumors*

Watanabe *et al*. firstly studied the relationship between cancer and Reg[16]. Reg I mRNA was detected at various levels in gastric cancer and colorectal cancer, but was not in esophageal cancers and nontumoral mucosae of the colon, rectum and esophagus. Reg gene family has been found to be up-regulated in human colorectal cancer cell lines during differentiation[50], this was reflected at the protein level by Western blotting in a small series of human colorectal cancers[14]. Macadam *et al*. later analyzed 142 cases of primary colorectal adenocarcinoma and demonstrated that 53 % tumors expressed Reg I mRNA, which was only detected in 16 out of 88 (18.1 %) paired normal mucosae[12]. PAP was also over-expressed in colorectal cancer[14]. Reg genes were expressed in a portion of cancers, whereas no expression was found in paired normal mucosae. The mechanisms altering the transcriptional control of Reg genes might be of interest from a therapeutic standpoint[12].

Even though there is a long history of observations related to up-regulated Reg expression in cancer. Only in the last five years, there have been experimental evidences directly supporting a role of these proteins in neoplastic transformation and tumor progression. Bernard-Perrone *et al*. localized Reg I protein in Paneth cells and immature columnar cells of human small intestinal crypts[50], which appeared to be associated with cell growth. Reg I protein may be down-regulated when growth is achieved and differentiation is induced.

Our study on Reg IV suggested that Reg IV might play an important role in initiating colorectal adenoma, and its detection might be useful in the early diagnosis of colorectal adenoma formation[8]. Reg IV has been screened 13 times in a subtracted cDNA library of human colon adenoma/normal mucosa by using suppression subtractive hybridization (SSH) method[8, 38]. The overexpression of Reg IV in colorectal adenomas was testified by reverse Northern blot. Our recent results showed that Reg IV was up-regulated in colorectal adenoma and carcinoma. Violette *et al*. also found its overexpression in colorectal cancer[10], and pointed out the potential role of Reg IV in colorectal tumors and its subsequent interest as a prognostic indicator.

Another interesting topic is involved in Reg I and gastric cancer. Reg I could be expressed in gastric enterochromaffin-like (ECL) cells[51,52]. Mutations of Reg I that inhibit secretion are associated with ECL cell carcinoids, suggesting that Reg I functions as an autocrine or paracrine tumor suppressor. Chiba suggested that Reg I might normally function as a negative regulator of ECL cell growth to restrain the stimulatory effect of gastrin in humans[53]. Abolition of Reg I protein secretion might result in an enhanced proliferation of ECL cells, and eventually lead to the development of ECL carcinoid tumors.

In addition to the study on relationships between diseases of gastrointestinal tract and Reg expression, there are many reports dealing with Reg and other digestive organs. Harada *et al*. examined the expression of Reg I in intrahepatic cholangiocarcinoma (ICC) and its precursor lesion (biliary dysplasia) and showed that the expression of Reg I protein was significantly dependent on the histologic differentiation[54]. HIP at a transcriptional level was elevated in liver tumors while it was not detected in nontumorous adjacent areas or in normal adult and fetal liver, suggesting that HIP could be involved in liver cell proliferation or differentiation. HIP mRNA expression is tissue specific, since it is expressed in the normal small intestine and pancreas, while it could not be evidenced in colon, brain, kidney, or lung[37]. HIP gene shows several potential regulatory elements, which might account for the enhanced expression of the gene during pancreatic inflammation and liver carcinogenesis[55]. Both Reg I mRNA and its product were localized in acinar cells of the pancreas, but neither was found in ductal or islet cells. Reg I protein has been considered as a useful marker for acinar cell differentiation[56] and immunohistochemical application of reg I protein may help to show histogenesis and differential diagnosis of pancreatic tumors.

Few reports are available on the association of Reg and tumors outside the digestive system. Bartoli *et al*. showed a weak expression of PAP/HIP gene in the pituitary gland, Reg I expression was not observed in tested adenomas or in pituitary gland[57], whereas REGL gene was observed in pituitary gland and in some subtypes of adenomas. Reg gene was expressed only in fetal pancreas and in some adult tissues. In contrast, REGL transcript was expressed not only in fetal pancreas but also in fetal colon and brain as well as in some adult tissues. In our results, we firstly reported Reg IV' s potential role in prostate cancer[8]. From the original results of bioinformatics analysis based on public databases (serial analysis of gene expression, SAGE), we could state that Reg IV was expressed in normal colon mucosa, colon adenocarcinoma, pancreatic cancer, gastric adenocarcinoma and prostate adenocarcinoma. Since Reg family was associated with different kinds of tumors in the digestive system, this was the report of Reg IV expression in prostate adenocarcinoma although its transcript level was rather low. On the other hand, if Reg is expressed in most cancers independent of their origins and is only expressed in limited normal tissues, it is of clinical significance that Reg expression is applied to early detection and treatment of cancer. But the most important point is to make sure whether its expression is tumor-specific.

## ASPECTS TO BE FURTHER STUDIED

### *Reg family and microsatellite instability*

Microsatellite instability (MSI) has been reported to be an important feature of solid malignancies[58]. Inactivation of the mismatch repair system (MMR) would lead to MSI that can profoundly affect cellular behaviors, since many genes playing important roles in inducing signal transduction, apoptosis, DNA repair and cell cycle control could be altered in tumors with MSI[59].

From the report of Akiyama *et al*.[30], we can hypothesize the possible relationship between Reg and DNA repair. Although it cannot supply strong evidences for the relationship between Reg and microsatellite instability, but other reports may further reveal its potential relationship. Cancers with MSI have a unique histological appearance and an altered response

to chemotherapy and radiotherapy. It has been more frequently seen in mucoid cancer with poor differentiation[60-64] and the subtype with MSI also has a characteristic of drug resistance. Interestingly, Violette *et al*. discovered that Reg IV mRNA-positive tumor cells displayed unique phenotypes such as: mucus-secreting, enterocyte-like or undifferentiated ones. What is more, it was overexpressed in HT-29 drug-resistant cells[10].

### Possible early biomarkers of carcinogenesis

Zenilman *et al*. observed a phenomenon of Reg I protein expression changed in colorectal cancer[9] and postulated that colorectal production of Reg I might either be a marker for the presence of cancer or a risk of mucosa for development of neoplasia based on the fact that Reg I protein was ectopically expressed in colorectal mucosa at the transitional zone of colorectal cancer, and occasionally within the tumor itself[9].

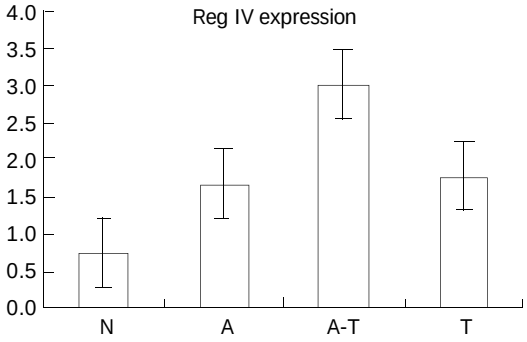


**Figure 1** *In situ* hybridization results of 12 cases of colorectal adenoma with carcinomatous change. (N=normal mucosa, A=residual adenoma, A-T=adenoma with carcinomatous change, T=invasive cancer)[13].

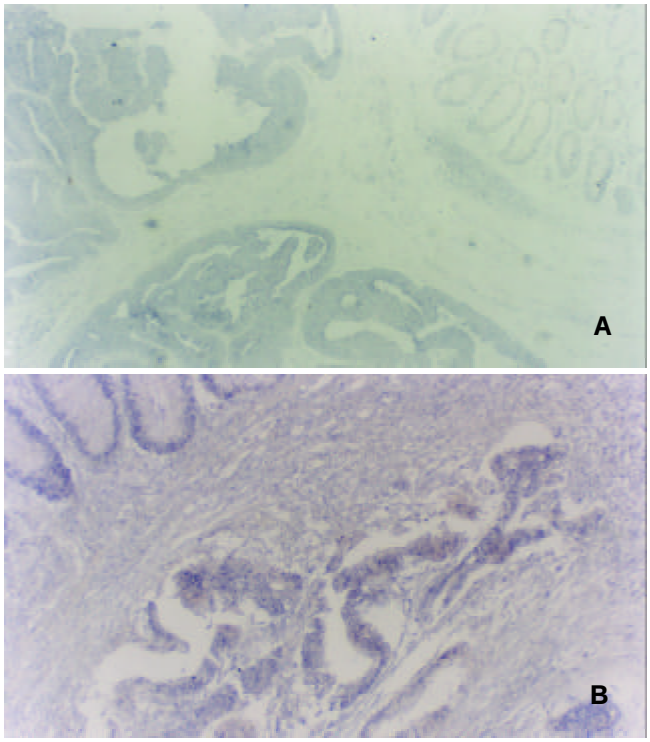


**Figure 2** *In situ* hybridization of Reg IV. A: Reg IV is up-regulated in colorectal cancer, B: Reg IV is up-regulated in normal mucosa adjacent to adenoma and adenocarcinoma.

Other reports on Reg family can add some evidences to the confirmation of Reg family' s role as a predicator of early cancer. Harada *et al*. suggested that expression of Reg I was a good marker for biliary mucosa at risk for development of ICC, and that Reg I played a role in the early stages of biliary carcinogenesis, probably via a cell-proliferative effect[54]. Our results of *in situ* hybridization showed that Reg IV was up-regulated in colorectal adenoma and carcinoma and the greatest Reg IV positivity was typically observed at regions with carcinomatous change (Figures 1, 2). Another interesting phenomenon was that at some regions near adenoma or adenocarcinoma (Figure 2b), Reg IV was also up-regulated as it was in colorectal adenoma and carcinoma, and the expression became weaker with increasing distance away from the tumor border in the direction of normal epithelia. Thus Reg IV may be thought as the biomarker of early transformations such as *in situ* carcinoma. This further presented evidences that overexpression of Reg IV might be an early event in colorectal adenoma-carcinoma sequence and carcinogenesis. Our present data also show that Reg IV is more frequently overexpressed in poorly differentiated colorectal carcinomas and carcinomas with metastasis.

The tumor-promoting activity of Reg protein should be considered for its possible clinical applications[43]. Moreover, its sensitivity to carcinogenesis might be used for early diagnosis.

### Antiapoptotic factors

Reg I might reduce epithelial apoptosis in inflammation[65,66]. In addition, tumor necrosis factor (TNF) pathway also reflects Reg' s potential role as an antiapoptotic factor. TNF-alpha contributes to the development of acute pancreatitis. Because TNF-alpha was involved in the control of apoptosis, Malka *et al*. studied its interaction with the pancreatic apoptotic pathway[65,66]. The antiapoptotic pancreatitis-associated protein (PAP) I is a candidate for mediating TNF-alpha activity. Its expression is induced by TNF-alpha, and cells overexpressing PAP I show significantly less apoptosis on exposure to TNF-alpha. Therefore, PAP I is one of the effectors of apoptosis inhibition.

Reg gene product may regulate a series of regeneration. This regenerative response may switch off apoptotic signals. Thus, those cells, which exhibit both a regenerative response and genetic mutations in some growth promoting or metastasis inducing genes, would have a survival advantage[12].

Up to now, we cannot confirm whether Reg I directly take part in apoptotic inhibition. But it is of interest to clarify the relationship between Reg I expression and inhibitors of apoptosis.

### Predictions of poor prognosis

Expression of Reg might reflect the degree of tissue injury. Thus overexpression degree of Reg might be a useful marker to judge whether the tumor has a poor prognosis. The expression of Reg I alone and co-expression of Reg I with PAP have a significantly adverse effect on survival. Thus the expression of Reg I might provide a valuable selective indicator of adjuvant therapy in patients with early-stage colorectal cancer which would recur after curative surgery[12].

Reports also revealed that a role of Reg gene in the healing of gastrointestinal mucosal lesions. Up-regulation of Reg I expression in ulcerative colitis might reflect the activation of mucosal injury followed by down-regulation when the injury was healed. Interestingly, a similar feature was also appeared in Reg IV. Our results showed that higher levels of Reg IV mRNA were consistently scored in regions with more severe dysplasia in the same adenoma sample displaying a varying degree of dysplasia. We postulate that Reg IV overexpression may reflect the degree of body injuries. However, when two tumors coexisted in a single case, Reg IV transcript was usually

higher in adenomas compared with paired carcinomas. The mechanism is not clear.

In addition to these mechanisms, Reg family has been found to be implicated in other physiological processes. Reg II has a distinctive role in injury response[67] and its expression is a crucial step in ciliary neurotrophic factor (CNTF) survival pathway. Reg II has been found to be a neurotrophic factor and also an intermediate in the survival signalling pathway of CNTF-related cytokines[68].

In conclusion, we have characterized the structure and expression of Reg genes. Their possible functions and mechanisms were also discussed. These studies have led to a better understanding of the essential functions of these Reg family genes in mammalian physiological processes and human diseases. In addition to its potential application in diabetes, future studies on Reg family and human malignancy will shed lights on Reg' s unusual features on cancer. As we know, expression of Reg I inversely correlated with the level of cell differentiation, and it could be modulated via the glucocorticoid receptor, and has been found to be a potential marker of gastrointestinal epithelial differentiation[69]. Rechreche *et al*. suggested that inhibition of PAP/reg expression in normal colon cells by silencing their gene promoters could be relieved during colon carcinogenesis, allowing their up-regulation by mediators such as cytokines. Reg' s role in human malignancy especially in the digestive system should be further studied[11,14]. More recently, Kamarainen *et al*. identified and characterized a gene encoding a regenerating protein (REG)-like protein called RELP[70], and found there were several transcripts of RELP and the predicted protein product of the major transcript was annotated Reg IV.

All these encourage us to further study the potential roles of Reg family, especially Reg IV in human tumors. Are they sensitive reactants of tissue injury? Do they play oncogenic roles in colorectal cancers? Is there any potential if they are used as early biomarkers of carcinogenesis? Could they be used as prognostic indicators of tumor survival and disease severity? Before these conclusions can be drawn, further investigations are needed. Thus, detection of the expression level of Reg in cancers with different histological features and survival rates, and analysis of Reg gene status in human tumors at different stages or sites, are required to elucidate the pathophysiologic roles of Reg family.

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