Dysregulation of Reg gene expression occurs early in gastrointestinal tumorigenesis and regulates anti-apoptotic genes

Kumar S. Bishnupuri  
*Washington University School of Medicine in St. Louis*

Qizhi Luo  
*Washington University School of Medicine in St. Louis*

Joshua R. Korzenik  
*Massachusetts General Hospital*

Jeffrey O. Henderson  
*Tabor College*

Courtney W. Houchen  
*University of Oklahoma Health Sciences Center*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

Please let us know how this document benefits you.

**Recommended Citation**

[https://digitalcommons.wustl.edu/open_access_pubs/3043](https://digitalcommons.wustl.edu/open_access_pubs/3043)

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Dysregulation of Reg Gene Expression Occurs Early in Gastrointestinal Tumorigenesis and Regulates Anti-Apoptotic Genes

Kumar S. Bishnupuri1
Qizhi Luo1
Joshua R. Korzenik3
Jeffrey O. Henderson4
Courtney W. Houchen5
Shirikant Anant5
Brian K. Dieckgraefe1,2

1Division of Gastroenterology and 2Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri USA
2Division of Gastroenterology; Massachusetts General Hospital, Boston, Massachusetts USA
3Department of Biology; Tabor College; Hillsboro, Kansas USA
4Department of Internal Medicine; University of Oklahoma Health Sciences Center; Oklahoma City, Oklahoma USA
5Department of Internal Medicine; University of Oklahoma Health Sciences Center; St. Louis, Missouri USA

*Correspondence to: Brian K. Dieckgraefe; Washington University; Department of Biology; St. Louis, Missouri USA

©2006 Landes Bioscience

E-publication: http://www.landesbioscience.com/journals/cb/article/3469

INTRODUCTION

Tumorigenesis is a multistep process involving somatic mutations or epigenetic changes affecting tumor suppressor and oncogenes.1,2 Additional genetic alterations create a permissive environment for clonal expansion of cells that are resistant to apoptosis. Advanced forms of common malignancies, such as colorectal, gastric, prostate or breast carcinoma are often associated with poor responses to adjuvant chemotherapy (CT) and/or ionizing radiation (IR).3 Apoptosis is a prominent mechanism for cell death following CT or IR.4 Accordingly, considerable attention has been given to the Bcl-2 family genes as possible regulators of intrinsic tumor resistance to therapy.5 Repressors of programmed cell death, such as Bcl-2 and Bcl-xL, decrease IR- and CT-induced apoptotic cell death in cell culture.6 However, key cellular factors that regulate expression of anti-apoptotic genes in tumors remain unknown. Defining dominant pathways responsible for modulation of apoptosis-regulating proteins would significantly enhance our understanding of tumor behavior and could broaden current strategies for therapeutic intervention.

The regenerating (Reg) genes constitute a family belonging to calcium dependent (C-type) lectin gene superfamilies.7-10 The Reg family genes included six members (Reg I, Reg II, Reg IIIa, Reg IIIb, Reg IIIc and Reg IIIγ) in the mouse and three members (Reg Iα, Reg Iβ and Reg III) in humans.11 Human Reg IV, a novel member of the family was identified by high throughput sequencing of a library derived from patients with ulcerative colitis, constituting fourth member of the Reg gene family in humans.10 A mouse homologue has also been identified constituting the seventh member in mouse. Expression of Reg genes is increased following injury, supporting a potential role in tissue repair and regeneration.12,13 Expression of Reg proteins by colorectal, gastric, and pancreatic adenocarcinomas have recently been shown to have an adverse association with patient survival.14-16 Reg IV was among several genes with increased expression in cancer cell lines selected for increased in vitro resistance to the chemotherapeutic agent, 5-FU.6 Reg IV expression was...
associated with intestinal differentiation in gastric adenocarcinoma and highly elevated in colorectal cancer (CRC). Reg IV has also been identified as a promising marker of hormone refractory metastatic prostate cancer. Recently, we observed the mitogenic effect of Reg IV protein, when added to the cultures of human colon adenocarcinoma cell lines with subsequent changes in expression of genes associated with altered apoptosis and metastasis. This supports the hypothesis that Reg gene products are responsible for altered apoptosis associated with a more aggressive tumor phenotype. Here we examined the expression of individual Reg genes in human colorectal adenocarcinoma specimens and adenomas from multiple intestinal neoplasia (Min) mice heterozygous for a germ-line nonsense mutation of the adenomatous polyposis coli (APC) gene. These animals spontaneously develop multiple polyps in the small and large intestine at 10–12 weeks of age following spontaneous second mutation in the APC gene. These results identify aberrant expression of the Reg genes as one of the earliest events in gastrointestinal tumorigenesis. Reg IV was specifically upregulated at the time of adenoma formation and contributed to the increased resistance to apoptotic cell death.

**MATERIALS AND METHODS**

**Cell lines and culture.** HCT116 and HT29 colon adenocarcinoma cells (American Type Culture Collection, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium (Cambrex, Walkersville, MD) containing 10% heat inactivated fetal bovine serum (Sigma, St. Louis, MO). Cells were placed in serum-free media overnight prior to treatment with endotoxin-free recombinant human recombinant interferon-γ (Sigma, St. Louis, MO). Cells were subjected to PAGE electrophoresis and blotted on PVDF membranes (Millipore, Bedford, MA).

**Human colorectal carcinoma specimens.** Five millimeter sections and total RNA isolated from human colorectal adenocarcinoma specimens and paired normal mucosa were obtained from the Tissue Procurement Core of the Siteman Cancer Center, Washington University.

**Animals.** Breeding pairs of C57Bl/6j APCmin/+ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were maintained on a 10% fat diet (Harlan Teklad, Madison, WI). Young APCmin/+ mice were genotyped as previously described.

**Immunohistochemistry.** Immunohistochemical staining of human colorectal adenocarcinoma specimens and adenoma isolates from APCmin/+ mice was performed by using previously characterized antibodies against Reg IV and Bcl-2 (Transduction laboratory, BD Biosciences, Franklin lakes, NJ) in the Digestive Disease Research Center Histopathology Core.

**Real time RT-PCR analysis.** Total RNA isolated from human colorectal adenocarcinoma isolates and paired normal mucosa, adenosas from APCmin/+ and their wild-type littersmates (APC+/+) and human colon adenocarcinoma cells (HCT116 and HT29) was converted to cDNA using SuperScript II reverse transcriptase and random hexanucleotide primers (Invitrogen, Carlsbad, CA). Samples were analyzed by real time RT-PCR using Jumpstart Taq DNA polymerase (Sigma, St. Louis, MO) and SYBR Green nucleic acid stain (Molecular Probes, Eugene, Oregon) or Taqman probes (IDT, Coralville, IA) for individual genes. Crossing threshold values for individual genes were normalized to GAPDH (murine) or β-Actin gene expression. Probe and primer sets designed for real time RT-PCR analysis are shown in Table 1.

**Western blot analysis.** Cell lysates from HCT116 and HT29 cells were subjected to PAGE electrophoresis and blotted on to Immobilon™-PVDF membranes.

**Table 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species*</th>
<th>Primer</th>
<th>Probe &amp; dye</th>
<th>dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>M</td>
<td>GGCATATCTCCACGGCAGCAGT</td>
<td>JOE/AGGCCGAGAATGGGAGCTTGTAC/</td>
<td></td>
</tr>
<tr>
<td>β Actin</td>
<td>H &amp; M</td>
<td>ATCATGTCTCCTCCTGAGGC (F) TGGTATCCACATCTGGTGGA (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Reg I</td>
<td>M</td>
<td>CATCTCTCTCTCATGCTTA (F) GCAGATGCGAGCTCCTCC (R)</td>
<td>TET/TGTCCTCCTAGCAGCAGCAGT/</td>
<td></td>
</tr>
<tr>
<td>Reg Ia</td>
<td>H</td>
<td>TATGACCTCTGCTGAGG (F) CCCTTCTGGGACGCTTCC (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Reg Ib</td>
<td>H</td>
<td>TCTGAGCGCTGCTGCTGCTG (F) GCCTTCTGGGAGCCTTCC (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Reg II</td>
<td>M</td>
<td>CACGACAGGCGGCGCGTTACTG (F) GGGCGATTGTGTTGCAGCAGA (R)</td>
<td>FAM/ACTCTCCCCCTGCTGAAAAGACCTTCC/</td>
<td></td>
</tr>
<tr>
<td>Reg III</td>
<td>H</td>
<td>GTAAACAGCTACCTCATACCTGGA TTG (F) CTCACCATCCTCCTATTGG (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Reg IIIa</td>
<td>M</td>
<td>GGATGAGCTCCCATGATGC (F) TCAGCAGACCTGAGTACTCCA (R)</td>
<td>FAM/CCTCCATGGGRTGGTGGACCCATTGT/</td>
<td></td>
</tr>
<tr>
<td>Reg IIIβ</td>
<td>M</td>
<td>TGGTGGCTCCCTGTCCCTTCTC (F) CAGCTGATGCGTGGGAAGAC (R)</td>
<td>TET/TGCCATGGGTAGCTTACCGGAC/</td>
<td></td>
</tr>
<tr>
<td>Reg IIIγ</td>
<td>M</td>
<td>GTGACAGATGCCTGAGGTTA (F) CCACCTCCTTGGGTGCATT (R)</td>
<td>TET/TGGGCCTACAGCCGAGACTG/</td>
<td></td>
</tr>
<tr>
<td>Reg IV</td>
<td>M</td>
<td>CGTCGCGCAGTCTCCTGCGT (F) AGCTGCTGCTTCAAGTAC (R)</td>
<td>FAM/CTGGGCGACTGCGCTGGCAC/</td>
<td></td>
</tr>
<tr>
<td>Reg IV</td>
<td>H</td>
<td>TGAGCTCGCTGGCCCAAA (F) AAGTACACGTCATCGTCTG (R)</td>
<td>TET/TGCCATGGGCTGCGCTGGCAC/</td>
<td></td>
</tr>
<tr>
<td>Bcl 2</td>
<td>M</td>
<td>TGGATCCAGGATAACGGAAG (F) CAAACAGAGGTCGCATGCTG (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Bcl 2</td>
<td>H</td>
<td>CCGTCTCTGGGTGCGCTAT (F) GAAAGCTCAGCGCCTGCCC (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
<tr>
<td>Bcl xl</td>
<td>H</td>
<td>CCCATGCTCCCTATCTCTG (F) TAAGTGCATCATCAGAGCTG (R)</td>
<td>SYBR</td>
<td>BHQ</td>
</tr>
</tbody>
</table>

*H, human; M, murine.
Reg IV is the dominant member of Reg gene family upregulated in human colorectal adenocarcinomas. (A) Absolute mRNA expression of Reg Iα, Reg Iβ, Reg III and Reg IV genes was determined in 14 surgically resected human colorectal adenocarcinomas specimens and paired normal mucosa. Expression level of individual Reg gene was normalized to 1000 arbitrary units of β-actin gene expression and plotted on logarithmic scale. Expression profile shows that Reg IV is the dominant member of Reg family expressed at higher level in normal mucosa and further increased in colorectal adenocarcinomas. (B) Relative to normal mucosa, fold change in expression of Reg genes was determined for individual colorectal adenocarcinomas specimens. 100%, 86%, 86% and 71% of colorectal adenocarcinoma specimens demonstrated increased Reg IV, III, Iα and Iβ expression respectively greater than 1.5-fold. (C) Immunohistochemical staining of adenocarcinoma isolates demonstrated increased expression of Reg IV protein. Representative sections demonstrate strong expression of Reg IV by a subset of cells with goblet-like morphology (left panel) in normal mucosa and staining by all cells (middle and right panels) in adenocarcinomas.

Antibodies were purchased from Transduction laboratory (BD Biosciences, Franklin Lakes, NJ) and Santa Cruz Biotechnology (Santa Cruz, CA). Specific proteins were detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Piscataway, NJ).

In vitro radiation-survival colony assay: HCT116 and HT29 cells were plated at 10^4 cells/25 cm^2 flasks and incubated in serum containing media overnight to allow cell adherence. Cultures were then incubated in serum free media with or without 100 nM rhR4. After 18 hours, cells were subjected to either 0 or 4 Gy γ-irradiation (IR) in a Gamacel 40 cesium irradiator at 0.96 cGy/min. Cells were allowed to grow until the development of microscopically visible colonies.

After 18 hours, cells were subjected to either 0 or 4 Gy (IR) in a Gamacel 40 cesium irradiator at 0.96 cGy/min. Cells were then incubated in serum free media with or without 100 nM rhR4. Reg IV was the dominant member of the Reg family expressed in normal mucosa (mRNA expression: 6.1 ± 4.5, 0.45 ± 0.2, 0.05 ± 0.03, and 27.2 ± 9.3 of Reg Iα, Reg Iβ, Reg III and Reg IV respectively) (Fig. 1A). While colorectal adenocarcinoma specimens exhibited increased expression of all Reg genes, Reg IV, and to a lesser extent Reg Iα constituted the dominant members of the Reg gene family expressed by colorectal adenocarcinomas (mRNA expression: 492 ± 304, 56 ± 33, 8 ± 6, and 1456 ± 676 of Reg Iα, Reg Iβ, Reg III and Reg IV respectively) (Fig. 1A). Individual resected tumors showed highly unique patterns of Reg gene expression. For example, specimen 3 had increased Reg IV expression only; however, specimen 2 had increased expression of all members of the Reg gene family including Reg IV. 100%, 86%, 86%, and 71% of colorectal adenocarcinoma specimens demonstrated increased Reg IV, III, Iα, and Iβ expression respectively greater than 1.5-fold relative to adjacent normal mucosa (Fig 1B). No clear correlation between levels of Reg gene expression and a particular histopathology or tumor stage was observed (data not shown). Consistent with increased Reg IV mRNA, colorectal adenocarcinomas showed prominent expression of Reg IV protein by immunohistochemistry (Fig. 1C).

Expression of Reg genes is dysregulated early in tumorigenesis. We first characterized the expression of the Reg gene family in the normal murine GI tract. Total RNA was isolated from individual segments of the GI tract, extending from the stomach to the colon of 14-week old mice. Reg gene expression was then determined by using Taqman probe and primer sets specific for each of the 7 Reg genes in the murine genome. Individual Reg genes displayed one of three embryologically-derived expression patterns across the cranio-caudal axis of the adult GI tract (Fig. 2A). Reg I, II and IIIβ had maximal expression in the foregut-derived stomach and duodenum; Reg IIIα, IIIβ, and IIIγ in mid-gut-derived small bowel extending from the jejunum to the ileum, whereas Reg IV was unique with prominent expression in the cecum and colon. We next utilized APC^min/+ mice to determine at what step expression of individual Reg genes becomes dysregulated during tumorigenesis. We analyzed regional expression of different Reg genes in the intestines of four-week old APC^min/+ mice and wild-type (APC^+/+) littermate controls. Four-week old wild-type APC^+/+ mice had regional expression patterns mirroring that shown in 14-week normal adults (data not shown). Compared to wild-type APC^+/+ mice, different intestinal segments of four-week old APC^min/+ mice showed significant increases in expression of all Reg genes, except Reg IV (Fig. 2B). Intestinal regions associated with the greatest predisposition for adenomatous polyp formation had increases of greater than two-fold in Reg I, II, IIIβ and IIIγ expression. Furthermore, segmental increases were seen in both Reg members expressed normally at that site (e.g., Reg IIIβ in the ileum).

Statistical Analysis. Values were expressed as the mean ± s.e.m. Data were analyzed by 2-tailed t test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Reg IV is most upregulated gene of Reg family in human colorectal adenocarcinomas. In order to determine Reg gene expression in gastrointestinal tumors, we measured the expression of human Reg genes in 14 colorectal adenocarcinoma resection specimens and paired normal mucosa. mRNA expression of individual Reg genes was determined by real time RT-PCR and normalized to β-actin expression. Absolute mRNA level of individual Reg gene was determined based on titrated standard curve using specific primer sets and probes. Reg IV was the dominant member of the Reg family expressed in normal mucosa (mRNA expression: 6.1 ± 4.5, 0.45 ± 0.2, 0.05 ± 0.03, and 27.2 ± 9.3 of Reg Iα, Reg Iβ, Reg III and Reg IV respectively) (Fig. 1A). While colorectal adenocarcinoma specimens exhibited increased expression of all Reg genes, Reg IV, and to a lesser extent Reg Iα constituted the dominant members of the Reg gene family expressed by colorectal adenocarcinomas (mRNA expression: 492 ± 304, 56 ± 33, 8 ± 6, and 1456 ± 676 of Reg Iα, Reg Iβ, Reg III and Reg IV respectively) (Fig. 1A). Individual resected tumors showed highly unique patterns of Reg gene expression. For example, specimen 3 had increased Reg IV expression only; however, specimen 2 had increases in expression of all members of the Reg gene family including Reg IV. 100%, 86%, 86%, and 71% of colorectal adenocarcinoma specimens demonstrated increased Reg IV, III, Iα, and Iβ expression respectively greater than 1.5-fold relative to adjacent normal mucosa (Fig 1B). No clear correlation between levels of Reg gene expression and a particular histopathology or tumor stage was observed (data not shown). Consistent with increased Reg IV mRNA, colorectal adenocarcinomas showed prominent expression of Reg IV protein by immunohistochemistry (Fig. 1C).
Reg Gene Expression in Tumorigenesis

as well as Reg genes not expressed at that location (e.g., Reg II and III). Further, to determine changes in Reg gene expression associated with adenoma development, visible adenomas and adjacent normal mucosa were microdissected from 14-week APCmin/+ mice. Cox-2 expression was used as a marker for successful adenoma isolation. Reg IV represented the most commonly increased member of the family (≥1.5 fold in 72% of adenomas) (Fig. 3). Similar expression profiles of individual Reg genes were also observed in each adenoma examined, mirroring human colorectal adenocarcinoma specimens.

Reg IV regulates anti-apoptotic genes. Increased Bcl-2 and Bcl-xL expression is a frequent occurrence in colon adenocarcinomas and is predictive of poor prognosis.

www.landesbioscience.com Cancer Biology & Therapy 1717
Reg IV treatment induces cell survival against radiation-induced apoptosis. Repressors of programmed cell death may directly increase resistance to therapy-induced cell death. We therefore investigated a possible protective role of Reg IV in human colon adenocarcinoma cells using an in vitro radiation-survival colony assay (Fig. 6). HCT116 and HT29 cells grown on culture plates containing media with or without 100 nM rhR4 were exposed to 4 Gy IR. The microscopically visible colonies in this model are reflective of a single surviving and proliferating cell. Exogenous Reg IV treatment significantly increased the number of colonies. Following 4 Gy IR, colony counts increased from $34.8 \pm 5.1$ to $50.4 \pm 3.5$ (45% increase, $p < 0.05$) in HCT116 (left panel) and
apoptotic cell death. It is likely that a variety of cellular pathways may
with an increased cell proliferation and an associated decrease in
In each of these models, it appears that tumorigenesis is associated
Fearon describes step-wise transformation from the benign polyp to
sion of Reg IV in colorectal carcinomas.
accordance with previously reported data showing elevated expres
upregulated member of the family in these specimens. This was in
observed increased expression of all genes of
an adverse association with patient outcomes or survival.
36.8 ± 3.5 to 60.3 ± 5.7 (64% increase, p<0.005) in HT29 cells (right panel). In the absence of IR, rhR4 treatment did not result in a
difference in the number of colonies. This data indicates that Reg IV
promotes tumor cell survival following a potent apoptotic stimulus.

**DISCUSSION**

Most adenocarcinomas are relatively resistant to CT and IR.\(^{25-27}\)
Efforts to overcome this resistance by increasing concentration of
cytotoxic drugs or dosage of irradiation has been failed to signifi-
cantly improve the therapeutic response. Apoptosis is a prominent
mechanism for death of cancer cells following CT or IR.\(^3,26\)
Novel cancer treatment strategies targeting the genes that promote apo-
tosis or blocking factors that inhibit apoptosis may prove adjuvant in
the treatment of many malignancies. The expression of the Reg gene
family is increased in common malignancies including colorectal,
gastric, hepatocellular and pancreatic adenocarcinomas and may have
an adverse association with patient outcomes or survival.\(^{14-17,28}\)
We observed increased expression of all genes of Reg family in a series of
human colorectal adenocarcinoma specimens. Reg IV was the most
upregulated member of the family in these specimens. This was in
accordance with previously reported data showing elevated expres-
sion of Reg IV in colorectal carcinomas.\(^{18,19}\)
The multistep tumorigenesis model proposed by Vogelstein and
Fearon describes step-wise transformation from the benign polyp to
the malignant phenotype of colorectal cancer.\(^{1,2}\) A similar multistep
process was also observed in a hepatocarcinogenesis model in rats.\(^{29}\)
In each of these models, it appears that tumorigenesis is associated
with an increased cell proliferation and an associated decrease in
apoptotic cell death. It is likely that a variety of cellular pathways may
contribute to decreased apoptosis at specific stages of tumorigenesis.
This may also be required for the development of a malignant pheno-
type. The APC\(^{\text{min/}+}\) mouse model was chosen, because it mimics the
developmental process of human GI tumorigenesis. These mice
spontaneously develop multiple adenomas in small and large intest-
tine at around 10–12 weeks of age following a second spontaneous
mutation in the APC\(_{\text{gene}}\). Compared to wild-type littermate controls
(APC\(^{+/+}\)), four-week old APC\(^{\text{min/}+}\) mice already had significant
increases in expression of Reg genes, preceding the second spontaneous
mutation in the APC\(_{\text{gene}}\). However, increased Reg IV expression
was not detected prior to the polyp formation or adenomatous
changes by histology. Significant increases in Reg IV expression
were noted in a series of adenomas microdissected from 14-week
old APC\(^{\text{min/}+}\) mice. This data is first of its kind to demonstrate that
the Reg IV might have a potential role during adenoma formation
following second spontaneous mutation in APC\(_{\text{gene}}\). These data also
demonstrated that adenoma formation was associated with increased
expression of at least one Reg gene, mirroring the results observed
in human colorectal adenocarcinomas. Our results in the APC\(^{\text{min/}+}\)
mouse model are in agreement with findings of a 2–3 fold increase
in Reg IV expression in flat colonic mucosa containing microscopic
adenomatous changes in three patients with familial adenomatous
polyposis (data not shown).

Increases in Reg IV expression in adenomas of 14-week old
APC\(^{\text{min/}+}\) mice had an associated increase in Bcl-2 expression.
Human colorectal adenocarcinoma specimens with increased Reg IV
expression exhibited increased expression of Bcl-2 and Bcl-x\(_L\) mRNA
(data not shown). A causative role for Reg IV in regulation of the
Bcl-2 family genes has been confirmed with increased expression of
Bcl-2 and Bcl-x\(_L\) at mRNA and protein levels following addition of
rhR4 to culture media of human colon adenocarcinoma cell lines.
This result supported our previous finding of increased expression
of anti-apoptotic genes via Reg IV-mediated EGFR/Akt signaling
for all.\(^{21}\) Furthermore, addition of rhR4 to human colon adeno-
carcinoma cells led to significantly greater resistance to cell death
following IR. Functional blocking of Reg IV protein increased cell
susceptibility to IR-induced death (data not shown). Collectively,
these data suggest that up-regulation of Reg IV and corresponding
changes in cellular predisposition to apoptosis may play a requisite
early role in adenoma formation.

In summary, individual Reg genes show a specific expression
profile along the canic-caudal axis of the GI tract. The expression of
Reg genes is increased in colorectal adenocarcinoma and constitutes
an early event in intestinal tumorigenesis in APC\(^{\text{min/}+}\) mice. While the
APC\(^{\text{min/}+}\) mouse model does not completely recapitulate human
colon cancer given the expression of polyps in both the small bowel
and colon, the model offers the unique opportunity to study genetic
changes occurring at a premalignant stage, before more profound
genetic derangements and clonal selection pressures. Increased
Reg IV expression might lead to a tumor phenotype displaying
increased resistance to apoptotic cell death. These results identify
Reg proteins as previously unappreciated regulators of anti-apoptotic
proteins in early tumorigenesis and may contribute to increased
resistance to apoptotic death during therapy. Strategies designed to
reduce endogenous Reg expression or block downstream signaling
warrant further investigation for use in the prevention or treatment
of established gastrointestinal adenocarcinomas.

**Figure 6.** In vitro radiation-survival colony assay showing decreases in
radiation-induced apoptosis following rhR4 treatment in human colon
adenocarcinoma cells. HCT116 (left panel) and HT29 cells (right panel)
were plated at 10\(^4\)cells/25 cm\(^2\) flask and incubated overnight to allow cell
adherence. Cultures were then treated with complete media with or without
100 nM rhR4. After 18 hours cells were subjected to either 0 or 4 Gy IR and
allowed to grow until the development of visible colonies. Colonies counted
reflects survival and proliferation of individual cells (lower panel). Addition
of rhR4 to HCT116 and HT29 cells significantly increased the number of
surviving cells following 4 Gy IR (A and C, p > 0.05, control vs. 4 Gy IR; B
and D, p < 0.05, 4 Gy IR vs. 4 Gy IR + rhR4 with HCT116 and HT29 cells
respectively).

www.landesbioscience.com Cancer Biology & Therapy 1719
References


15. Macadam RC, Sarela AI, Farmery SM, Robinson PA, Markham AF, Guillou PJ. Death from early colorectal cancer is predicted by the presence of transcripts of the REG gene family. Br J Cancer 2000; 83:188-95.


