Glucagon-like peptide-2 increases dysplasia in rodent models of colon cancer

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Submitted 2 December 2011; accepted in final form 2 February 2012

Trivedi S, Wiber SC, El-Zimaity HM, Brubaker PL. Glucagon-like peptide-2 increases dysplasia in rodent models of colon cancer. Am J Physiol Gastrointest Liver Physiol 302: G840–G849, 2012. First published February 9, 2011; doi:10.1152/ajpgi.00505.2011.—The intestinal hormone, glucagon-like peptide-2 (GLP-2), enhances intestinal growth and reduces inflammation in rodent models. Hence, a degradation-resistant GLP-2 analog is under investigation for treatment of Crohn’s disease. However, GLP-2 increases colonic dysplasia in murine azoxymethane (AOM)-induced colon cancer. Considering the increased colon cancer risk associated with chronic colitis, we have therefore examined the effects of long-acting hGly2GLP-2, as well as of endogenous GLP-2 using the antagonist hGLP-23–33 in two novel models of inflammation-associated colon cancer: rats fed the carcinogen 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and a high-fat diet (HFD) for one or three cycles, and mice with chronic dextran sodium-sulfate (DSS)-induced colitis administered AOM. hGly2GLP-2 treatment of one-cycle PhIP/HFD rats increased the number of colonic aberrant crypt foci by 72 ± 11% (P < 0.01). Fifty-one weeks after three PhIP/HFD cycles, hGly2GLP-2-treated rats had a 22% incidence of colon cancer, compared with 0% in vehicle-treated rats. AOM-DSS mice treated with vehicle or hGly2GLP-2 had high-grade dysplasia/colon cancer incidences of 56 and 64%, respectively, compared with 46% in hGLP-23–33-treated AOM-DSS animals (P < 0.05). Unexpectedly, hGLP-23–33 also reduced the colitis damage score by 32.0 ± 8.4% (P < 0.05). All high-grade dysplastic/cancerous tumors had nuclear localization of β-catenin although β-catenin mRNA transcript and protein levels did not differ between treatment groups. GLP-2 receptor mRNA expression also was not different. However, hGLP-23–33-treated mice had markedly reduced numbers of doublecortin- and calmodulin-kinase-like-1-positive stem cells, by 73.7 ± 8.6% (P < 0.05). In conclusion, the results of this study indicate a role for hGly2GLP-2 and endogenous GLP-2 as potential cancer promoters in rodents. AOM-DSS mice have an inactivating mutation in the APC gene, which prevents degradation of β-catenin, whereas Apcmin/+ mice have an inactivating mutation in the adenomatous polyposis coli (APC) gene, which prevents degradation of β-catenin (6, 36, 40, 50, 55, 59). To gain further insight into the broader applicability of GLP-2 as a potential colon cancer promoter, we have now examined the effect of hGly2GLP-2 on colonic dysplasia and cancer in two novel models of inflammation-associated colon cancer, including a second species, rats, administered another carcinogen, the dietary heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in association with a high-fat diet (HFD), as well as mice administered AOM in the setting of chronic dextran sulfate sodium (DSS)-induced colitis.
MATERIALS AND METHODS

Animals

Adult (5–6 wk), male Fischer 344 (F344) rats and adult (6–10 wk), male C57BL/6 mice were purchased from Charles River Canada (Charles River Canada, St. Constant, QC, Canada). All animals were housed in a facility with a 12-h:12-h light/dark cycle and given ad libitum access to water and food. All experimental protocols were approved by the Animal Care Committee of the University of Toronto.

Experimental Protocols

PhIP-ACF study. A rat model of dietary carcinogen-induced pre-neoplastic colonic lesions, detected as aberrant crypt foci (ACF), was developed based on the protocol in Ref. 45 (Fig. 1A). In brief, all rats were fed regular AIN-93G powdered chow diet with 16.8% fat-derived calories from soybean oil for 2 wk (Dyets, Bethlehem, PA), either alone or mixed with 400 ppm of the common dietary carcinogen (34), PhIP (Toronto Research Chemicals, North York, ON, Canada). Food intake and body weight were measured on alternate days during this period. This was followed by feeding of all rats for 4 wk of HFD with 59.2% fat-derived calories, obtained by supplementing AIN-93G diet with Primex (hydrogenated vegetable oil, Dyets), such that 41.3% of calories were derived from Primex and 17.9% from soybean oil. Feeding of a HFD is known to be associated with induction of mild intestinal inflammation (16, 35). During weeks 3 and 4 of the study, the rats were injected with either 50 mM ammonium bicarbonate (Bicarb, 200 μl sc bid; vehicle control; n = 4 for rats without PhIP feeding; n = 8 for rats with PhIP feeding) or hGly2GLP-2 (40 μg, 200 μl sc bid; American Peptide, Sunnyvale, CA; n = 8). During weeks 5 and 6 of the study, the dose of hGly2GLP-2 was increased to 60 μg to account for the increase in body weight. The rats were weighed twice weekly during weeks 3–6. The use of hGly2GLP-2 in all of the studies presented in this study precludes differences in DPP-IV levels (Bicarb, 200 μl sc bid), or hGLP-23–33 (30 ng in 200 μl sc bid; American Peptide); peptide doses were selected based on a previous validation study in normal mice treated with AOM (26). On the day of death, the mice were administered with vehicle, hGly2GLP-2, or hGLP-23–33 according to their respective groups 3 h prior to death. Small intestinal weight, colon weight, and colon length were obtained after gentle cleaning. Sections (0.5–2 cm in length) from the jejunum (5–10 cm proximal from the mid-small intestine) were fixed in 10% neutral buffered formalin. Any tumors found were photographed and dissected, and pieces were fixed in 10% neutral buffered formalin or frozen on dry ice for storage at −80°C. The total number and size of tumors could not be determined because the growths merged into large continuous masses in the majority of animals (not shown). Because of the large number of animals involved in this study, this experiment was performed using three separate cohorts: set 1 (all 3 treatment groups; n = 8–10 per group), set 2 (vehicle and hGly2GLP-2 treatments only; n = 18–19 per group), and set 3 (vehicle and hGly2GLP-2 treatments only; n = 9 for vehicle and n = 10 hGly2GLP-2-treated groups). Weekly body weights were recorded throughout the study. The animals were also monitored for signs of colon cancer development such as weight-loss and rectal bleeding and were killed if deemed necessary by veterinarians at the animal facility. All remaining animals were killed after week 61 of the experimental protocol. After death, the small intestine, cecum, and large intestine were removed and examined for signs of macroscopic lesions, which were photographed in situ and then dissected intact, fixed in formalin, sectioned, and stained with hematoxylin and eosin (H&E) for tumor classification.

AOM-DSS study. A murine model of CRC was developed on the basis of a modified protocol from Refs. 42 and 48. In brief, mice were injected with AOM (10 mg/kg ip; Sigma-Aldrich Canada, Oakville, ON, Canada) and allowed to recover for 1 wk (Fig. 1C). At the beginning of week 2, they were given 2.5% DSS in drinking water for 1 wk, followed by 2 wk of recovery. This cycle was performed three times. The dose of DSS was determined following a pilot study in which morbidity and colonic damage score (CDS) were assessed in mice treated with 1–3% DSS alone; an intermediate dose of DSS was selected based on the findings (data not shown). During the DSS cycles, mice were monitored daily for signs of morbidity, dehydration, and blood in stools. Rodent chow was mixed with regular water to create a mash in cases of severe dehydration. In contrast to the pilot study, the concentration of DSS administered had to be reduced or eliminated in some mice during the third cycle as a result of rectal bleeding, likely consequent to the prior administration of AOM; these mice were randomly distributed between the subsequent treatment groups, and the reduction in DSS did not affect the CDS in tissues collected upon completion of the study regardless of the treatment group. Mice were weighed weekly and were then randomized to one of three treatment groups after the last day of DSS treatment, with each treatment lasting for 2 wk: vehicle (50 mM Bicarb, 200 μl sc bid), hGly2GLP-2 (1.5 μg in 200 μl sc bid), or hGLP-23–33 (30 ng in 200 μl sc bid; American Peptide); peptide doses were selected based on a previous validation study in normal mice treated with AOM (26). On the day of death, the mice were administered with vehicle, hGly2GLP-2, or hGLP-23–33 according to their respective groups 3 h prior to death. Small intestinal weight, colon weight, and colon length were obtained after gentle cleaning. Sections (0.5–2 cm in length) from the jejunum (5–10 cm proximal from the mid-small intestine), ileum (5-cm proximal from the ileocecal valve), and colon were frozen on dry ice or fixed in 10% neutral buffered formalin. Any tumors found were photographed and dissected, and pieces were fixed in 10% neutral buffered formalin or frozen on dry ice for storage at −80°C. The total number and size of tumors could not be determined because the growths merged into large continuous masses in the majority of animals (not shown). Because of the large number of animals involved in this study, this experiment was performed using three separate cohorts: set 1 (all 3 treatment groups; n = 8–10 per group), set 2 (vehicle and hGly2GLP-2 treatments only; n = 18–19 per group), and set 3 (vehicle and hGly2GLP-2 treatments only; n = 9 for vehicle and n = 10 hGly2GLP-2-treated groups). Weekly body weights were measured on alternate days during this period. This was followed by feeding of all rats for 4 wk of HFD with 59.2% fat-derived calories, obtained by supplementing AIN-93G diet with Primex (hydrogenated vegetable oil, Dyets), such that 41.3% of calories were derived from Primex and 17.9% from soybean oil. Feeding of a HFD is known to be associated with induction of mild intestinal inflammation (16, 35). During weeks 3 and 4 of the study, the rats were injected with either 50 mM ammonium bicarbonate (Bicarb, 200 μl sc bid; vehicle control; n = 4 for rats without PhIP feeding; n = 8 for rats with PhIP feeding) or hGly2GLP-2 (40 μg, 200 μl sc bid; American Peptide, Sunnyvale, CA; n = 8). During weeks 5 and 6 of the study, the dose of hGly2GLP-2 was increased to 60 μg to account for the increase in body weight. The rats were weighed twice weekly during weeks 3–6. 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GLP-2 ENHANCES COLONIC DYSPLASIA

To determine the effects of GLP-2 on the development of ACF, in a novel species treated with a unique carcinogen, rats were acutely fed the colon cancer-inducing agent, PhIP, and then subjected to a HFD (Fig. 1A). Compared with vehicle-treated rats fed the control AIN-93G diet alone (i.e., NoPhIP+Bicarb group), rats fed with the PhIP diet had reduced gain in body weight over the 6-wk experiment (266 ± 0.05). However, normalized small intestinal and colon weights, as well as crypt and villus lengths were similar between the NoPhIP+Bicarb and PhIP+Bicarb animals, indicating that PhIP feeding per se did not have any effect on parameters of intestinal growth (Fig. 2, A–C). In contrast, whereas administration of hGly2GLP-2 did not alter
body weight gain among the PhIP-fed animals, exogenous hGly2GLP-2 led to a 67.4 ± 4.5% (P < 0.001) and 43.0 ± 6.3% (P < 0.001) increase in normalized small intestinal weight and villus height, respectively, without any changes in crypt depth (Fig. 2, A and B). There was also a trend toward increased colon weight with hGly2GLP-2 treatment although this did not reach significance (Fig. 2C). Together, these findings indicated that the hGly2GLP-2 utilized was biologically active in PhIP-fed rats.

Although a few ACF were detected in the NoPhIP/Bicarb rats, animals in the PhIP/Bicarb group had a 470 ± 106% increase (P < 0.01) in the number of ACF, compared with the NoPhIP/Bicarb animals (Fig. 2D). Moreover, PhIP-fed rats treated with hGly2GLP-2 demonstrated an even greater increase in the number of ACF, by 72 ± 11%, compared with rats in the PhIP/Bicarb group (P < 0.01). This increase was associated with enhanced numbers of one-crypt ACF (P < 0.05), but not of two- and three-crypt ACF (Fig. 2E). The distribution of ACF along the length of the colon was not different between the treatment groups, nor were there any differences in the number of mucin-depleted foci (data not shown).

**Rat PhIP-Tumor Study**

To evaluate the progression of ACF to cancer, rats were subjected to repeated cycles of PhIP and a HFD, followed by a HFD for an additional 42-wk period (Fig. 1B). By the 52nd wk of the study, three PhIP/HFD rats were found dead or were euthanized due to significant weight loss or other morbidity. Among these, at necropsy, one rat from the Bicarb-treated group was found with a noncancerous intestinal tumor, whereas a second died due to large-cell lymphocytic leukemia, both as assessed by the veterinarian of the animal facility. One hGly2GLP-2-treated rat died in association with excessive bleeding from the mouth and anus that was not further assessed. Analysis of tumor sections from the rats that survived to the end of study revealed that none of the PhIP+Bicarb-
treated rats had large intestinal cancer (i.e., 0 of 7 rats), whereas 22% of the rats given the hGly2GLP-2 treatment had cancerous tumors in the cecum or colon (i.e., 2 of 9 rats; Fig. 2F).

**Mouse DSS-AOM Study**

To determine whether the effects of AOM on colon cancer are modified in a model of increased risk, mice were treated with DSS for three cycles to mimic the setting of chronic colitis (Fig. 1C). A slight decrease in body weight was noted after each DSS cycle; however, body weights on the day of death were not different for the vehicle-, hGly2GLP-2-, and hGLP-23–33-treated groups (27.0 ± 0.3 g for Bicarb vs. 26.7 ± 0.3 g for hGly2GLP-2 and 27.8 ± 0.3 g for hGLP-23–33). Administration of hGly2GLP-2 significantly increased the normalized small intestinal weight by 48.8 ± 6.0% (P < 0.001; Fig. 3A), as well as jejunal villus height and crypt length of mice by 42.8 ± 3.5% and 10.1 ± 2.4%, respectively (P < 0.01–0.001; Fig. 3B). In contrast, blocking the actions of endogenous GLP-2 by administration of hGLP-23–33 did not alter normalized small intestinal weight, villus height, or crypt depth (Fig. 3, A and B). Finally, whereas administration of hGly2GLP-2 increased jejunal crypt proliferation at positions 2 and 20 (P < 0.05–0.01), administration of hGLP-23–33 reduced proliferation in positions 10, 14, 16, 17, and 19 (P < 0.05–0.01; Fig. 3C). Together, these results indicate that both the hGly2GLP-2 and hGLP-23–33 were biologically active in the murine DSS-AOM mouse model.

All colon tissue sections with suspected tumors were assessed for the presence or absence of low- or high-grade dysplasia and colon cancer, using both H&E- and AE1/AE3-stained sections. Whereas 56% of vehicle-treated (control) animals were not modified in a model of increased risk, mice were treated with DSS for three cycles to mimic the setting of chronic colitis (Fig. 1C). A slight decrease in body weight was noted after each DSS cycle; however, body weights on the day of death were not different for the vehicle-, hGly2GLP-2-, and hGLP-23–33-treated groups (27.0 ± 0.3 g for Bicarb vs. 26.7 ± 0.3 g for hGly2GLP-2 and 27.8 ± 0.3 g for hGLP-23–33). Administration of hGly2GLP-2 significantly increased the normalized small intestinal weight by 48.8 ± 6.0% (P < 0.001; Fig. 3A), as well as jejunal villus height and crypt length of mice by 42.8 ± 3.5% and 10.1 ± 2.4%, respectively (P < 0.01–0.001; Fig. 3B). In contrast, blocking the actions of endogenous GLP-2 by administration of hGLP-23–33 did not alter normalized small intestinal weight, villus height, or crypt depth (Fig. 3, A and B). Finally, whereas administration of hGly2GLP-2 increased jejunal crypt proliferation at positions 2 and 20 (P < 0.05–0.01), administration of hGLP-23–33 reduced proliferation in positions 10, 14, 16, 17, and 19 (P < 0.05–0.01; Fig. 3C). Together, these results indicate that both the hGly2GLP-2 and hGLP-23–33 were biologically active in the murine DSS-AOM mouse model.

**DISCUSSION**

GLP-2 is an intestinal hormone with a multitude of beneficial effects that lead to improved intestinal growth and function (51). Hence, long-acting analogs of GLP-2 are now being considered as treatment options for patients with intestinal insufficiency or inflammatory bowel disease (IBD) (8, 11, 29). The growth-promoting actions of both exogenous and endogenous GLP-2 in the intestinal mucosa are of particular concern in context of the finding that individuals with IBD are at increased risk of developing colon cancer (21, 52). Moreover,
it has also been shown that patients with active IBD have greater levels of endogenous bioactive GLP-2 (67) although this does not appear to be the case in patients with mild IBD (54). However, studies in the literature examining the effect of GLP-2 on colon cancer have used only mice to date, and only in models of sporadic colon cancer (26, 32, 63). Hence, to examine the broader applicability of GLP-2 as a potential cancer promoter, the effect of GLP-2 on dysplasia and cancer in the colon was studied herein using both a different species and a novel inducer, namely rats fed the carcinogen PhIP in association with a HFD, which not only induces mild intestinal inflammation, but also promotes the carcinogenic effect of PhIP (16, 34, 35, 41, 45). Of note, PhIP is a common dietary carcinogen found in cooked meat and fish that has been estimated to contribute to nearly 50% of the cancer risk associated with normal consumption of these foods (34). Moreover, to assess the effect of GLP-2 on colon cancer in association with more severe, chronic colonic inflammation, DSS-AOM was used to create a murine model of colon cancer-associated colitis. Together, these models cover two species with dietary and chemical carcinogenesis, in association with mild dietary- and more severe chemically-induced intestinal inflammation, respectively. When taken together with findings of a previous study in normal mice treated with AOM (26), the present results indicate that both exogenous and endogenous GLP-2 increase colonic dysplasia and, possibly, colon cancer in a variety of rodent models of this disease.

The major finding of this study was that, in rats fed PhIP, treatment with the long-acting GLP-2 analog hGly2GLP-2 enhanced dysplastic colonic growth. Although this change occurred predominantly at the level of one-crypt ACF, which are considered to be less dysplastic than larger ACF (60), it corresponded with the finding of colon cancer only in the PhIP-HFD rats treated with hGly2GLP-2. Similar findings were made in the AOM-DSS mice, such that the incidence of high-grade dysplasia and cancer was increased by exogenous GLP-2, whereas the development of these growths was decreased by GLP-2R antagonism. The changes in colon cancer incidence were relatively small in the present study, ranging from 22% in the rats to 15–17% (for exogenous and endogenous GLP-2, respectively) in the mice. Nonetheless, the findings are in accord with our previous observation of a 30–63% increase in ACF in AOM-mice treated with hGly2GLP-2 for 4 wk and a 22–39% decrease following administration of hGLP-23–33 with mucin-depleted foci and colon cancer found in the hGly2GLP-2-treated animals only (26). Similar effects have also been observed in mice administered the carcinogen dimethylhydrazine, wherein treatment with both GLP-2 and hGly2GLP-2 increased the number of colonic polyps (63). Although these findings add PhIP to AOM and dimethylhydrazine as a dysplastic agent for which the effects are modifiable by GLP-2 treatment, the results are difficult to reconcile with the findings of another study using APCmin/H11001 mice in which GLP-2 was not found to alter the incidence of adenomas (32). The major difference between the PhIP, AOM, and dimethylhydrazine rodent models of colon cancer and the APCmin/H11001 mouse is the driving agent for the abnormal growth, such that PhIP, AOM, and dimethylhydrazine are all models of sporadic colon cancer compared with the genetically driven small and large intestinal tumorigenesis caused by the APCmin/+ mutation (14). When taken together, therefore, these findings suggest that, although both exogenous and endogenous GLP-2 can enhance dysplastic growth in the rodent colon, the effects are dependent on the model utilized. These findings may also have clinical implications for patients treated with long-acting analogs of GLP-2, such that more frequent screening for colonic dysplasia may be preemptive.
Fig. 5. Effect of hGly2GLP-2 and hGLP-23–33 on colonic damage, GLP-2R expression, doublecortin-and-calmodulin-kinase-like-1 (DCAMKL-1)-positive stem cells and β-catenin levels in AOM-DSS mice. Normalized colonic weight (A; n = 25–31), colon length (B; n = 25–32), colonic damage score (CDS) (C; n = 26–34), GLP-2R mRNA transcript levels (D; n = 5–9), β-catenin mRNA transcript levels (E; n = 8–9 for normal tissue; n = 22 for high-grade dysplasia/cancer), β-catenin protein levels (F; n = 5 for normal tissue; n = 5 for high-grade dysplasia/cancer; a representative blot is shown) are shown. Representative β-catenin staining in normal tissue and high-grade dysplasia/cancer (G) and number of DCAMKL-1-positive stem cells (H; n = 10–14; a representative photomicrograph is shown) in mice treated with Bicarb (open bars), hGly2GLP-2 (shaded bars), or hGLP-23–33 (solid bars; *P < 0.05 as indicated) are shown.
tion, β-catenin accumulation, and stem cell growth were examined. Unexpectedly, although hGly2GLP-2 treatment has been shown to increase growth and reduce CDS in models of acute colitis, including that induced by DSS (19, 28, 33, 57), treatment with hGly2GLP-2 did not change these parameters in the setting of AOM-induced cancer with chronic colitis. Indeed, blocking endogenous GLP-2 action actually reduced CDS in this model. However, the model used in the present study was one of, not only repeated cycles of inflammation, but also cancer induction. It is therefore not clear whether the findings can be extended to humans with IBD, who have been shown in one trial to respond favorably to treatment with a long-acting GLP-2 analog (11). Nonetheless, the reduction in CDS corresponded with the finding of decreased carcinogenesis in the hGLP-23–33-treated mice and may have been a contributing factor, given the established link between inflammation and colon cancer risk (21, 52).

In contrast to the findings with CDS, no significant changes in GLP-2R mRNA transcript levels were found between the treatment groups. However, analysis of mRNA transcript levels may not reflect GLP-2R protein levels, and there are currently no commercially available antisera with which these can be ascertained. Therefore, β-catenin expression was analyzed as an indirect measure of GLP-2R signaling in the tumors. The Wnt pathway is known to be involved in tumorigenesis of human cancers and is a target for mutations in both AOM- and PhIP-induced carcinogenesis (15, 27, 36, 59, 61). Moreover, previous studies by Dubé et al. (20) have shown that GLP-2 activates β-catenin signaling in small intestinal crypt cells. Hence, we investigated the effects of hGly2GLP-2 and hGLP-23–33 on β-catenin mRNA expression and both protein expression and localization. Although the mRNA and protein levels of β-catenin were found to be similar in all treatment groups, immunohistochemistry revealed that there were higher levels of nuclear β-catenin in the tumor tissues. This result is consistent with the finding that β-catenin accumulation in colonic tumor tissues is a result of mutations in the β-catenin (Ctnnb1) and APC genes that prevent the ubiquitination and degradation of β-catenin (46). Notwithstanding, none of these findings appear to account for the findings of altered dysplasia and cancer rates in mice consequent to either increased or decreased GLP-2 activity.

Previous studies have demonstrated that intestinal crypt stem cells, not only regulate normal intestinal growth and repair, but also can play a role in tumorogenesis (3, 39). The two main populations of stem cells are the rapidly cycling crypt-based columnar cells, expressing Lgr5, and the quiescent stem cells found at position 4, identified by staining for DCAMKL-1 (4, 38). It has previously been reported that the DCAMKL-1-positive stem cells can be modulated by intestinal growth factors, such as gastrin, as well as by the presence of carcinogenic mutations (31, 37). Our finding that neither hGly2GLP-2 nor GLP-23–33 altered the numbers of these cells in the normal colon indicates that the growth effects of GLP-2 in the normal gut may not be mediated by increasing the prevalence of this population of stem cells although alterations in the cell cycle length cannot be precluded (53). Nonetheless, in high-grade dysplasia and cancer tissues, a marked reduction in DCAMKL-1-positive stem cells was observed in response to blockade of endogenous GLP-2, in association with reduced cancer incidence, suggesting a possible role for these cells in the actions of endogenous GLP-2 as a promoter of colitis-associated cancer.

Finally, it remains unclear as to the exact mediator by which the cancer-promoting effects of both endogenous and exogenous GLP-2 are mediated. Previous studies have demonstrated that the intestinal growth effects of exogenous GLP-2 are exerted indirectly through ErbB ligands and receptors, insulin-like growth factor-1 and its receptor, and keratinocyte growth factor (51), of which both the ErbB and insulin-like growth factor pathways have been implicated in the development of colon cancer (13, 23). Whether this is also the case for endogenous GLP-2, the relative potencies of the two peptides in the promotion of colon cancer, and the direct effects of these mediators of GLP-2 action on intestinal stem cells remains to be determined.

Collectively, the results of these studies demonstrate that hGly2GLP-2 promotes the development of preneoplastic lesions in rats fed with the dietary carcinogen PhIP, in support of previous findings made with a different carcinogen (i.e., AOM) in another species (i.e., murine; Ref. 26). Although only a small role for GLP-2 as a cancer promoter was identified in PhIP-fed rats and mice with AOM-induced colitis-associated cancer, blocking endogenous GLP-2 action was found to correspondingly reduce cancer, in association with factors that are known to drive dysplastic growth, such as colitis-associated colonic damage (a measure of inflammation) and stem cell numbers (3, 22, 44, 66). Although there have been no reports to date of intestinal dysplasia in patients with short bowel treated for up to 2 yr with long-acting analogs of GLP-2, or in those with Crohn’s disease treated for 8 wk, it has been suggested that such individuals may benefit from preventative surveillance (11, 30, 43). The results of the present study are clearly consistent with this recommendation, particularly for those with an intact colon who are treated chronically and/or have increased cancer risk in association with IBD. Furthermore, because blocking endogenous GLP-2 reduced colitis damage score as well as DCAMKL-1-positive cancer stem cells, these findings also suggest that hGLP-23–33 may be useful as a potential cancer-preventing biological agent in patients at increased risk for colon cancer.

ACKNOWLEDGMENTS

The authors are grateful to Dr. K. Banks, University of Toronto, for veterinary assistance and to Mr. A. Izzo for technical assistance.

GRANTS

S. Trivedi was supported by a graduate studentships from the Canadian Institutes of Health Research (CIHR; Frederick Banting and Charles Best Canada Graduate Scholarship) and the Department of Physiology, University of Toronto. S. Wiber was supported by summer studentships from the Canadian Association of Gastroenterology/CIHR and the Department of Physiology/University of Toronto Research Opportunity Program. P. Brubaker was supported by the Canada Research Chairs program. This work was supported by operating grants from the Canadian Institutes of Health Research (MOP-9940 and NMD-94732).

DISCLOSURES

P. Brubaker has received consulting fees from NPS Pharmaceuticals.

AUTHOR CONTRIBUTIONS

Author contributions: S.T. and P.L.B. conception and design of research; S.T. and S.C.W. performed experiments; S.T., S.C.W., H.M.E.-Z., and P.L.B. analyzed data; S.T., S.C.W., and P.L.B. interpreted results of experiments; S.T.
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