The hexosamine biosynthetic pathway and \( O\)-GlcNAcylation drive the expression of \( \beta\)-catenin and cell proliferation

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Submitted 29 July 2011; accepted in final form 21 November 2011


First published November 22, 2011; doi:10.1152/ajpendo.00390.2011.

The short half-life protooncogene \( \beta\)-catenin acquires a remarkable stability in a large subset of cancers, mainly from mutations affecting its proteasomal degradation. In this sense, colorectal cancers (CRC) form a group of pathologies in which early steps of development are characterized by an aberrant expression of \( \beta\)-catenin and an uncontrolled proliferation of epithelial cells. Diet has long been described as an influence in the emergence of CRC, but the molecular events that link metabolic disorders and CRC remain elusive. Part of the explanation may reside in hexosamine biosynthetic pathway (HBP) flux. We found that fasted mice being force-fed with glucose or glucosamine leads to an increase of \( \beta\)-catenin and \( O\)-GlcNAcylated levels in the colon. MCF7 cells possessing intact Wnt/\( \beta\)-catenin signaling heavily expressed \( \beta\)-catenin when cultured in high glucose; this was reversed by the HBP inhibitor azaserine. HBP inhibition also decreased the expression of \( \beta\)-catenin in HT29 and, to a lesser extent, HCT116 cells. The same observation was made with regard to the transcriptional activity of \( \beta\)-catenin in HEK293 cells. Inhibition of HBP also blocked the glucose-mediated proliferation capacity of MCF7 cells, demonstrating that glucose affects both \( \beta\)-catenin expression and cell proliferation through the HBP. The ultimate element conducting these events is the dynamic posttranslational modification \( O\)-GlcNAcylation, which is intimately linked to HBP; the modulation of its level affected the expression of \( \beta\)-catenin and cell proliferation. In accordance with our findings, we propose that metabolic disorders correlate to CRC via an upregulation of HBP that reverberates on high \( O\)-GlcNAcylation levels including modification of \( \beta\)-catenin.

glucose; hexosamine biosynthetic pathway; glutamine:fructose-6-phosphate amidotransferase; protooncogene; diet; colorectal cancers

THE WNT/\( \beta\)-CATENIN PATHWAY is fundamental during embryogenesis and for the renewal of the intestinal crypt epithelium (25). During these processes, the main component of this pathway, the protooncogene \( \beta\)-catenin controls cell proliferation by initiating the transcription of its target genes, including cyclin D1 and c-Myc (15, 29). This process is shut down by targeting \( \beta\)-catenin to the 26S proteasome. Wnt/\( \beta\)-catenin signaling is also crucial for cardiac and neuronal development and plays an important part in many disorders affecting these tissues such as cardiac hypertrophy and neurodegenerative diseases. Misregulations in this system are also often observed in hepatoblastoma, hepatocellular carcinoma, and ovarian or pancreatic cancers, but these dysfunctions were more accurately described in colorectal cancers (CRC). Eighty percent of CRC result from genetic alterations of the crucial member of the \( \beta\)-catenin destruction complex adenomatous polyposis coli (APC), preventing the proteasomal degradation of \( \beta\)-catenin (33) that acquires an aberrant stability. This leads to uncontrolled cell proliferation (27). Remarkably, over time, diet appears to be a key factor involved in CRC emergence. Some nutrients like carbohydrates are known to play a critical role in this process, since high-carbohydrate consumption greatly increases the probability of setting up CRC (10, 16). Moreover, some observations have drawn a correlation between CRC and diet; thus, CRC are also considered obesity-associated diseases. Since the 1990s, it has been clearly demonstrated that patients suffering from CRC show intolerance to glucose and resistance to insulin, reflecting a metabolic disorder (24, 31). Type 2 diabetes individuals also have a higher risk of developing CRC than normal individuals (17).

Despite these observations, the molecular mechanism linking a metabolic disorder or overfeeding to CRC remains unknown. To tentatively answer this question, we started with the observation that metabolic syndrome and diet lead to a common event, a punctual or permanent hyperglycemia. On the basis of their dependence on nutritional status, we questioned whether the hexosamine biosynthetic pathway (HBP) and the posttranslational modification (PTM) \( O\)-GlcNAcylation could contribute to the emergence of CRC (13). Two decades ago, it was determined that the development of insulin resistance requires three key components: glucose, insulin, and glutamine, and that a small percentage (2–3%) of glucose is used to provide UDP-GlcNAc (N-acetylglucosamine), the final product of the HBP (21). Other nutrients are also implicated in the makeup of this nucleotide sugar: ketogenic amino acids, glutamine, fatty acids, and sugars (hexoses). UDP-GlcNAc is the donor of the GlcNAc group for glycosylation processes, including \( O\)-GlcNAcylation. Accordingly, both UDP-GlcNAc and \( O\)-GlcNAcylation are considered nutritional sensors. Thanks to its privileged position among thousands of PTMs, \( O\)-GlcNAcylation reflects the cell’s nutritional status to the regulation of fundamental biological processes: transcription, translation, cell signaling, intracellular trafficking, cell cycle, and development (35). Thereby, an imbalance in the use of nutrients will have a repercussion in the homeostasis of the tissues, and consequently, pathologies such as cancers may arise. Numerous factors involved in the cancerization processes or cell cycle regulation are \( O\)-GlcNAcylated: the protooncogenes c-Myc, (7) and \( \beta\)-catenin (19, 26, 37), the tumor suppressor p53 (32), and components of the MAPK or PI3K pathways (8, 34). Last, the modification of \( O\)-GlcNAcylation...
levels in malignancy and tumoral progression has been identified as crucial to the development of breast, lung, liver, and colon cancers (11, 22, 36). In this study, using refeed or glucose/glucosamine force-fed mice, we made the correlation between an increase in β-catenin content and O-GlcNAcylation in the colon. We also report a similar observation in vitro by culturing MCF7 cells in a high-glucose medium.

In contrast to glucosamine, asazemine, the inhibitor of the rate-limiting enzyme of the HBP, affects glucose-induced β-catenin expression, its transcriptional activity, and cell proliferation in a dose-dependent manner. Elevation of the O-GlcNAcylation content, using the OGA inhibitor NButGT, increases the level of β-catenin and slows down the cell proliferation. The expression of β-catenin was diminished in the colorectal cancer cell line HT29 and slightly in HCT116 by azasemine and another HBP inhibitor, DON [5-oxo-6-diazo-\( \text{L-serine} \)] at 100 \( \mu \text{M} \). Moreover, we observed higher levels of β-catenin, OGT, OGA, and O-GlcNAcylation in these cells compared with a fetal colon line. Taken together, our observations and results led us to propose that diet and metabolic disorders are predisposition factors for cancers, particularly CRC, by promoting an elevation of β-catenin level and a stimulation of cell proliferation through the HBP.

MATERIALS AND METHODS

Cell culture and transfection. MCF7, HEK293, HT29, and HCT116 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing various concentrations of glucose (0, 1, or 4.5 g/l) for MCF7 and HEK293. CCD841CoN cells were maintained in Eagle’s minimum essential medium (EMEM). All cell lines were maintained in a medium supplemented with 10% (vol/vol) fetal calf serum (heat inactivated for the HT29 and the HCT116 cells), 2 mM L-glutamine, 5 IU/ml penicillin, and 50 μg/ml streptomycin at 37°C in a 5% (vol/vol) CO₂-enriched humidified atmosphere.

For a TOP/FOP Flash reporter assay, HEK293 cells were transfected with β-galactosidase, TOP-Flash, FOP-Flash, and β-catenin-2×Flag vector by the Lipofectamine 2000 (Invitrogen) reagent (2 μg) in six-well plates with 0.2 μg of DNA for 24 h.

shRNA plasmids (29-mer) were purchased from CliniSciences and were used according to the manufacturer’s indications.

Drugs. Glucosamine (GlcNH₂) was used at a final concentration of 20 mM; NBuTG (1,2-dideoxy-2′-propyl-α-β-glucopyranosyl-[2,1-D]-Δ-2′-thiazoline) at 100 μM; asazemine [O-(2-diazoacetyl)-L-serine] at 50 μM or at the indicated concentration (see text for details); and DON at 10 or 50 μM. Cells were treated for 16 h with the different drugs except as specified in the text.

Lysis and immunoprecipitation. Cells were first washed with 10 ml of cold phosphate-buffered saline (PBS) and lysed on ice with lysis buffer [10 mM Tris·HCl, 150 mM NaCl, 1% Triton X-100 (vol/vol), 0.5% sodium deoxycholate (wt/vol), 0.1% sodium dodecyl sulfate (SDS; wt/vol), and proteases inhibitors, pH 7.4]. Cell extracts were then centrifuged at 20,000 g for 10 min at 4°C. Supernatants were first precleared with Sepharose-labeled protein A for 1 h. After the beads were discarded, the supernatants were incubated together with the rabbit polyclonal anti-β-catenin (H102, Santa Cruz Biotechnology) at a final dilution of 1:500 and placed overnight at 4°C. Antibody-bound proteins were recovered after adding 30 μl of Sepharose-labeled protein A for 1 h at 4°C. Beads were then centrifuged for 1 min and subsequently washed with the following buffers: lysis buffer, lysis buffer supplemented with 500 mM NaCl, lysis buffer-TNE [10 mM Tris·HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4 (vol/vol)] and finally with TNE alone.

SDS-PAGE, western blotting, and antibody staining. Equal amounts of extracted protein were subjected to Western blotting. Samples were analyzed by 8, 10, or 15% reticulated SDS-PAGE under reducing conditions, and proteins were electroblotted on a nitrocellulose sheet (GE Healthcare). Efficiency of the transfer and equal loading were verified using Ponceau red staining. Membranes were first saturated for 45 min with 5% (w/v) nonfat acid milk in Tris-buffered saline (TBS)-TWEEN buffer [15 mM Tris-HCl, 140 mM NaCl, and 0.05% Tween 20 (vol/vol), pH 8.0]. Mouse monoclonal anti-O-GlcNAc (RL2, Ozyme) was used at a dilution of 1:1,000. Rabbit polyclonal anti-β-catenin H102 (Santa Cruz Biotechnology), and chicken anti-OGA (345, generously provided by Prof. G. W. Hart) were used at a dilution of 1:2,000. Mouse monoclonal anti-tubulin (Santa Cruz Biotechnology), rabbit polyclonal anti-histone 2B (Millipore), and rabbit polyclonal anti-GAPDH (Abcam) were used at a dilution of 1:5,000.

Membranes were incubated with the different antibodies overnight at 4°C and then washed three times with TBS-Tween for 10 min and incubated with either an anti-rabbit or an anti-mouse horseradish peroxidase-labeled secondary antibody at a dilution of 1:10,000 for 1 h. Finally, three 10-min washes were performed with TBS-Tween, and detection was carried out with enhanced chemiluminescence (GE Healthcare). Densitometry analyses of the Western blots were done with GeneTools software (version 3.07.03, Syngene).

Fractionation. Fractionation was realized with a Proteoextract subcellular proteome extraction kit (MERCK) as recommended by the manufacturer.

Luminescence assay. After transient transfection of Super8×TOPFlash(M50) or Super8×FOPFlash(M51) with β-galactosidase (determination of transfection efficiencies) and β-catenin2×Flag vectors in six-well plates, HEK293 cells were lysed using 200 μl of passive lysis buffer (Promega). Luciferase reporter activity was measured in triplicate in a 96-well plate following back-addition of 100 μl of Luciferase assay buffer (Promega). In parallel, 70 μl of Galacton Substrate Mix (1:200 Tropix Galacton; 99:200 Tropix Galacto reaction buffer diluent, 100:200 18 MΩ water; Applied Biosystems) was incubated for 20 min out of light, and 100 μl of Tropix Accelerator-II (Applied Biosystems) was back-added before β-galactosidase activity was measured.

Proliferation assay. MCF7 cells (2 × 10⁵) were cultured in 96-well plates using DMEM over 5 days. Each day, cell growth was determined using the MTS reagent method (Promega) according to the manufacturer’s directions.

In vivo assay. Eight- to twelve-week-old male C57BL/6Cr mice were purchased from the provider Charles River Elevage (Saint-Germain sur l’Arbresle, France). Procedures were carried out according to French guidelines and as described (12) for the care of experimental animals. Mice were adapted to the environment for 1 wk prior to study and maintained in a 12:12-h light-dark cycle with water and regular diet (65% carbohydrate, 11% fat, and 24% protein). For the experiment, mice were fasted for 24 h or refed a regular diet for 18 h (glucose was added to the drinking water at a concentration of 200 g/l after the fasted period). For gavage experiments, 24-h-fasted mice received glucosamine (2.5 g/kg) or glucose (5 g/kg) orally, and were killed 3 or 6 h after intraperitoneal anesthesia (pentobarbital sodium). Colons were then collected, washed in PBS, and lysed in 2 ml of NP-40 lysis buffer [10 mM Tris·HCl, 150 mM NaCl, 1% Tween 20 (vol/vol), 0.084% (wt/vol) sodium pyrophosphate, 0.018% (wt/vol) sodium orthovanadate, 1 μM PUGNAc [O-(2-acetamido-2-deoxy-D-gluco-pyranosylidene) amino-N-phenylcarbamate] and proteases inhibitors, pH 7.4]. Tissues were broken using UltraTurrax, incubated with gentle agitation for 2 h, and centrifuged at 20,000 g for 30 min at 4°C to take over the soluble fraction. Glycemia was measured using an Accu-Chek Performa apparatus (Roche).
RESULTS

Glucose or glucosamine force-feeding modulates β-catenin expression and O-GlcNAcylation level in vivo. To gain insight into the underlying mechanism of how feeding reverberates on the expression of β-catenin, C57BL6 mice were fasted for 24 h and then refed a regular diet for 18 h or force-fed with glucose or glucosamine for 3 or 6 h. The mice’s blood glucose concentrations were measured (Fig. 1A), and then colons were collected, homogenized, and analyzed by Western blotting (Fig. 1B). In refed and especially in force-fed mice, we observed increased β-catenin expression and elevation of the O-GlcNAcylation contents. Interestingly, it must be noted that the use of glucosamine impacted the β-catenin and the O-GlcNAcylation levels (Fig. 1B) without affecting mice glycemia (Fig. 1A).

We next questioned whether the expression and activity of β-catenin depended on the nutritional status and, more particularly, glucose concentration. To check this hypothesis, in vitro experiments were performed in MCF7 cells, which express a high amount of β-catenin without exhibiting any mutation in the Wnt/β-catenin pathway. The expression of β-catenin was determined according to the cell glucose status in vitro. MCF7 cells were grown in media containing increasing amounts of glucose for 24 h, and the expression of β-catenin was determined (Fig. 1C). β-Catenin and O-GlcNAcylation levels were maximal when glucose was used at 25 mM vs. 0 and 5 mM (Fig. 1C), correlating with the observation made in vivo (Fig. 1B). Interestingly, but not surprisingly, we noted that the proliferation rate of cells (MTS assays for 96 h) was accelerated according to the glucose status (Fig. 1D). These data indicate that the expression of β-catenin correlates with the status of glucose both in vivo and in vitro.

Expression of β-catenin and its transcriptional activity are dependent on HBP flux. After entering the cell, glucose follows different metabolic pathways: glycogen synthesis, pentoses shunt, glycolysis, or the HBP, which provides UDP-GlcNAc, the substrate for the O-GlcNAcylation processes (Fig. 2A). Owing to the quicker expression of β-catenin after administration of glucose and glucosamine to fasted mice, we thought that glucose/glucosamine influenced the expression of the protooncogene by being harnessed through the HBP. This pathway was explored using azaserine, an inhibitor of its rate-limiting enzyme GFAT (glutamine:fructose-6-phosphate amidotransferase) (Fig. 2A). MCF7 cells were maintained in a culture medium containing three different concentrations of glucose with or without azaserine. We observed an increase of β-catenin expression (Fig. 2B) and transcriptional activity (TOP/FOP-Flash assays; Fig. 2C) following high-glucose

Fig. 1. β-Catenin and O-GlcNAcylation levels are elevated in refed and force-fed mice compared with fasted mice. C57BL6 mice were fasted for 24 h and refed a regular diet for 18 h or force-fed with glucose or glucosamine for 3 or 6 h. A: glycemia of each mice used in this study was measured, and values were represented as a histogram. B: colon homogenates were analyzed by immunoblot according to their β-catenin, GAPDH, and O-GlcNAcylation contents. The ratios of β-catenin/GAPDH expressions for the different conditions are represented as a histogram. C: MCF7 cells were cultured with 0, 5.5, or 25 mM glucose for 24 h, and expression of β-catenin and level of O-GlcNAcylation were determined by Western blot. D: proliferation rate of cells was determined for each glucose concentration condition by using the MTS method at 490 nm (n = 6). WB, Western Blot. Molecular mass markers are indicated at the left (in kDa).

*P < 0.05, **P < 0.01.
treatment, and, as expected, azaserine reversed the glucose effect at both the expression and the activity levels of β-catenin. This result demonstrates that the glucose-mediated β-catenin expression is conducted by the HBP. Since the functions of β-catenin depend on its subcellular localization, subcellular fractionation was performed on MCF7 cells following azaserine treatment (Fig. 2D). The glucose concentration upregulated the expression of the protooncogene in the cytosol and the nucleus (the same results were obtained by culturing cells with glucosamine or the OGA inhibitor PUGNAc; data not shown) without affecting the distribution, and azaserine reversed this enhancement in the two fractions. From this set of experiments, it can be deduced that the HBP flux increases the expression...
and activity of β-catenin without modifying its subcellular distribution. It has been extensively reported that stabilization of β-catenin affects cell proliferation via transcription of its target genes (15, 29). Finally, after we observed that cell proliferation was dependent on the concentration of glucose (Fig. 1D), we tested increasing concentrations of azaserine on the proliferation capacities of MCF7 cells (Fig. 2E). These experiments show that the proliferation rate of the cells is blocked by the use of azaserine in a dose-dependent manner. DON was also used in proliferation rate assays and showed that, similar to azaserine, it reduced cell proliferation (Fig. 2E, inset). Through these findings, we demonstrated that glucose enhances the expression of β-catenin and its transcriptional capability through the HBP without modifying its subcellular distribution. We also demonstrated that MCF7 cell proliferation is controlled by the HBP flux.

HBP enhances β-catenin expression and accelerates cell proliferation by elevating O-GlcNAcylation levels. As depicted in Fig. 2A, UDP-GlcNAc is the main end product of the HBP. This nucleotide sugar is the donor of the GlcNAc group for all glycosylation processes, including the O-GlcNAcylation. It was demonstrated that plakoglobin, which follows a process of degradation similar to that of β-catenin, is protected by O-GlcNAcylation (14). Therefore, we suggested that the fate of β-catenin was linked to the same PTM. To check this hypothesis, MCF7 cells were incubated with increasing amounts of glucose and then incubated with NButGT, a specific inhibitor of OGA, azaserine, or glucosamine that short-cuts the HBP by bypassing GFAT (Fig. 2A). Interestingly, both NButGT and glucosamine elevated the expression of β-catenin (Fig. 3A). As we observed with glucose (Fig. 1D) and azaserine and DON (Fig. 2E), we tested the effect of NButGT on the proliferation rate of MCF7 cells (Fig. 3B). As expected, and contrary to

Fig. 3. Expression of β-catenin and proliferation rate of MCF7 cells are dependent on O-GlcNAcylation status. A: MCF7 cells were cultured in DMEM using various concentrations of glucose. The OGA inhibitor NButGT, the HBP activator glucosamine, and the GFAT inhibitor were tested at the indicated concentration for 18 h. Cell homogenates were immunoblotted with anti-β-catenin, anti-O-GlcNAc (with and without free N-acetylglucosamine), and anti-tubulin antibodies. B: MCF7 cells were grown in DMEM supplemented with 25 mM glucose with or without NButGT for 5 days. The proliferation rate of the cells was determined for each day by using the MTS method at 490 nm (n = 6). C: MCF7 cells were incubated with the protein synthesis inhibitor cycloheximide (CHX), NButGT, or both for indicated time periods. Expression of β-catenin and actin were analyzed by Western blot. Efficiency of NButGT was confirmed using an anti-O-GlcNAc antibody. D: MCF7 cells were transfected with a shRNA plasmid for 24 h to specifically decrease expression of OGT. A scrambled construction was used as a negative control. Expressions of OGT, O-GlcNAcylation, β-catenin, and GAPDH were thus determined by Western blot. W/O, without NButGT (vehicle). Molecular mass markers are indicated at the left (in kDa).
azaserine and DON, the OGA inhibitor accelerated the proliferation of the cells. In another set of experiments, MCF7 cells were incubated with cycloheximide (CHX) with or without NButGT. The \(\beta\)-catenin content was then assessed for increasing time periods (Fig. 3C). We observed that the inhibition of OGA compensates for the effect of CHX on the expression of \(\beta\)-catenin. These results demonstrated that O-GlcNAcylation prevents the degradation of \(\beta\)-catenin. Last, we used a shRNA strategy to knock down the OGT (Fig. 3D). We observed that the downexpression of the glycosyltransferase also downregulated the level of \(\beta\)-catenin.

\(\beta\)-catenin and O-GlcNAcylation levels are elevated in colorectal cancer cell lines compared with normal cells. Azaserine and DON were both used in MCF7 as well as HT29 (adenocarcinoma) and HCT116 (carcinoma), two colon cancer cell lines expressing high amounts of \(\beta\)-catenin (Fig. 4A) (33). The two inhibitors were efficient in MCF7 and HT29 and to a lesser extent in HCT116 cells. These differences may be explained by the fact that HCT116 cells express a \(\beta\)-catenin mutated in the D-box (\(\Delta S45\)) that dramatically decreases its phosphorylation and impedes its correct targeting to the proteasome. The last point of the study compared the expression of \(\beta\)-catenin, the O-GlcNAc cycling enzymes, and the level of O-GlcNAcylation in HT29 and HCT116 cells with a normal colon cell line, CCD841CoN (21 wk gestation fetus) (Fig. 4B). \(\beta\)-Catenin, OGT, OGA, and the PTM are heavily expressed in the cancer cell lines. To measure the level of O-GlcNAcylation of the \(\beta\)-catenin in the three cell lines, the protooncogene was immunoprecipitated and analyzed according to its O-GlcNAcylation content (Fig. 4B). Interestingly, we showed a direct correlation between the modification and the expression of

Fig. 4. \(\beta\)-Catenin, O-GlcNAc cycling enzymes, and O-GlcNAYlation levels are elevated in colorectal cancer cell lines. A: efficiency of HBP inhibitors azaserine and DON was determined in HT29 and HCT116 compared with MCF7 cells. B: HT29, HCT116, and CCD841CoN cells were analyzed according to their \(\beta\)-catenin, O-GlcNAcylation, OGA, and OGT contents by immunoblot (left). The O-GlcNAcylated status of each cell line’s \(\beta\)-catenin was checked by analysis of protooncogene immunoprecipitates with anti-O-GlcNAc antibody (right). Controls of immunoprecipitation (IP) were performed using nonrelevant rabbit IgG antibodies. Molecular mass markers are indicated at the left (in kDa).

Fig. 5. Hypothetical mechanism by which diet and uncontrolled cell proliferation are linked. An excess of nutrients including carbohydrates elevates the production of UDP-GlcNAc, the end product of the HBP and substrate of OGT. Among the plethora of targets of OGT, \(\beta\)-catenin acquires an aberrant stability (it escapes proteasomal degradation) and therefore increased transcriptional activity, the cell gains an uncontrolled proliferation that may lead to cancer.
β-catenin. This last observation strengthens our hypothesis, according to which O-GlcNAcylation level β-catenin expression and emergence of cancer could be linked.

**DISCUSSION**

β-Catenin mutations are found in a plethora of human tumors: desmoid, endometrial, gastric, hepatic, ovarian, pancreatic, and colorectal. A defect in Wnt signaling is found in various human diseases, including osteoarthritis, Dupuytren’s disease, polycystic kidney disease, and cardiovascular and neurodegenerative diseases. During their lifetimes, nearly 5% of individuals will develop a CRC, which, among the 200 forms of cancer, ranks first in terms of morbidity and mortality. The progression of CRC is a long process that is strictly organized spatially and temporally according to Vogelstein’s sequence (9). During the succession of events, crucial components, such as oncogenes and tumor suppressors belonging to diverse signaling pathways, accumulate mutations (18). In 90% of CRC cases, mutations occur in the Wnt/β-catenin pathway, constituting early steps in the cancerization process of the colic and rectal mucosa (18, 27). In this way, inactivating mutations of the tumor suppressor APC are found in 85% of the sporadic forms of CRC, whereas 10% of these cancers possess activating mutations in β-catenin itself (23). Beyond the hereditary character of CRC and of the first importance played by environmental factors, it appears that the sedentary, overfed Western lifestyle enhances the risk of CRC emergence (2, 3, 5). Indeed, over time, the metabolic syndrome has been described as promoting CRC (6, 10, 17, 24). It has been established that an imbalance in food uptake and energy expenditure is associated with the development of CRC; this problem is more remarkable for obese and type 2 diabetes individuals (4–6). It is also noticeable that nutrients like carbohydrates are more influential for the progression of cancer (10, 16), but what links carbohydrate excess to CRC remains to be deciphered, even though the setting up of insulin resistance may be part of the explanation (24). Accordingly, nutritional metabolic troubles could have repercussions on the deregulation of the HBP and thus O-GlcNAcylation processes (Fig. 5), resulting in cell signaling failures, with Wnt signaling being directly affected in CRC.

Understanding and apprehending the underlying mechanisms causing CRC in response to a metabolic disorder should help reduce the progression of this serious health issue. β-Catenin and its associated signaling pathway are early actors in the development of CRC. The functions of β-catenin are intimately linked to its short half-life, its synthesis, and its degradation being rigorously controlled (33). Unfortunately, exposed mutations in the machinery regulating its expression can cause dramatic effects (18, 25, 27). β-Catenin is modified by numerous PTMs (30), including phosphorylation and ubiquitination (20), that control targeting of the oncoprotein to the 26S proteasome, and O-GlcNAcylation, whose function has been poorly studied (19, 26, 27). Owing to the homology between β-catenin and plakoglobin (14), and according to our observations, we propose that β-catenin acquires an aberrant stability, i.e., an enhanced half-life, after it is modified by the OGT (Fig. 5). This lifestyle-dependent stabilization may precede or aggravate the subsequent mutations affecting the Wnt/β-catenin pathway. Since a part of glucose participates in the production of UDP-GlcNAc, the substrate of OGT, we suggest that diet and CRC are linked through the HBP. Stabilization of β-catenin by activation or mutation of the Wnt pathway promotes the acceleration or loss of control of cell proliferation. The second case may lead to CRC. Previous studies already pointed out the crucial role of glucose in the expression of β-catenin (1, 28) and are in perfect agreement with our findings. Those authors showed that, in macrophages, glucose increases the expression of β-catenin in an HBP-dependent manner. Here, we found that, in addition to upregulating Wnt signaling by autocrine activation as demonstrated by Anagnostou and Sheperd (1), the HBP also stabilizes β-catenin by favoring its O-GlcNAcylation; these two consequences may be exerting a synergistic effect. We therefore propose that an elevated flux through the HBP, and accordingly an elevated O-GlcNAcylation status, constitutes a new process in the cancerization of the colic and rectal mucosa and explain why metabolic disorders and overfeeding enhance the risk of CRC.

**ACKNOWLEDGMENTS**

We are indebted to Dr. David Vocadlo (Simon Fraser University, BC, Canada), who provided us NbGUTG, and to Dr. Randall T. Moon (University of Washington, Seattle, WA), who provided us the TOP/FOP flash system. We are also grateful to Heather N. Russell-Simmons (Markey Cancer Center, University of Kentucky, Lexington, KY) for proofreading the manuscript.

**GRANTS**

This work was financially supported by the Ligue Contre le Cancer/Comité du Nord, the University of Lille 1, and the Centre National de la Recherche Scientifique. S. Olivier is a recipient of a fellowship from the Ministère de l’Enseignement Supérieur et de la Recherche.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: S.O., C.G., and T.L. conception and design of experiments; S.O., C.G., and T.L. data collection; S.O. and T.L. analysis and interpretation of data; S.O. and T.L. preparation of the manuscript; S.O. and T.L. manuscript final version; T.L. approved final version of manuscript; T.L. edited and revised manuscript.

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