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Promotion of adenoma growth by dietary inulin is associated with increase in cyclin D1 and decrease in adhesion proteins in Min/+ mice mucosa

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Abstract

We have earlier shown that dietary fructo-oligosaccharide inulin enhances adenoma growth in multiple intestinal neoplasia (Min/+) mice. To further explore inulin-induced early biochemical changes in the normal-appearing mucosa, Min/+ mice were fed from the age of 5 weeks to the ages of 8 and 15 weeks a control diet or an inulin-enriched diet (10% w/w). In addition, the wild-type littermates were fed with the same diets until the age of 8 weeks, in order to determine whether similar changes happen both in the wild-type and Min/+ mice. The mucosa without adenomas was collected and fractionated to nuclear, cytosolic and membrane pools. The protein levels of β-catenin, cyclin D1 and E-cadherin were determined by Western blotting at both time points, and immunohistochemical stainings were done for 8-week-old mice. The promotion of adenoma growth by inulin (week 15, 1.3-fold increase, $P=.0004$) was associated with accumulation of cytosolic and nuclear β-catenin, and increased amount of cytosolic cyclin D1 (1.5-fold increase, $P=.003$) in the normal-appearing mucosa of the Min/+ mice. Furthermore, inulin feeding reduced the membranous pools of β-catenin and E-cadherin. Also in the wild-type mice the drop in membranous β-catenin was clear ($P=.015$), and, moreover, a subset of crypts had enhanced nuclear β-catenin staining. These data indicate that dietary inulin can already activate in the normal-appearing mucosa β-catenin signaling, which in the presence of *Apc* mutation induces adenoma growth and even in the wild-type mice direction of the changes is similar.

Keywords: Adhesion; Cyclin D1; Intestinal tumorigenesis; Inulin; Min mouse

1. Introduction

The development of colon cancer is a long process that takes decades. Diet can both prevent and induce colon carcinogenesis, for instance, through epigenetic changes, which regulate the homeostasis of the intestinal mucosa. Food-derived compounds are constantly present in the intestine and may shift cellular balance toward harmful outcomes such as increased susceptibility for mutations.

One of the first attacks against the normal intestinal mucosa is the *APC* gene mutation that is also found in nearly all colon cancer cases [1]. The animal model of colon cancer — multiple intestinal neoplasia (Min/+) mouse — has a heterozygous mutation in the *Apc* gene leading to the truncated Apc protein and to the development of numerous intestinal adenomas [2,3]. One of the important functions of the Apc is the regulation of the Wnt signaling pathway [4,5]. One functioning *Apc* allele is enough to regulate Wnt signaling pathway, but the loss of both *Apc* alleles causes cellular hyperproliferation and subsequent adenoma growth. This regulation is linked to the β-catenin, as Apc is a part of the cellular β-catenin destruction complex. The lack of well-functioning Apc protein interrupts β-catenin destruction; β-catenin accumulates in the cytosol and is transferred to the nucleus. In the nucleus, it activates the transcription of the Tcf/Lef transcription factor mediated genes, e.g., cyclin D1 [6,7] and c-myc [8].

In the normal healthy mucosa, β-catenin is mainly located in the membranes, where it is part of the adherence junctions and links actin cytoskeleton to transmembrane protein E-cadherin [9]. Loosening of the adherence junctions, e.g., by the destruction of E-cadherin–β-catenin complexes, may disturb cellular balance and may be one of the very early events in the process of adenoma formation. The reduced levels of membrane β-catenin accelerate tumor progression but its reduction from the

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membranous pool is also reported to be among the earliest alterations in intestinal tumorigenesis [10–12]. The loss of E-cadherin expression or function is an important reason for the disruption of tight epithelial cell–cell contacts leading to the tumor formation [13].

One of the important β-catenin target proteins is cyclin D1, which is linked directly to the cell proliferation — it is one of the key molecules regulating the progression of cell cycle from G1 to S phase [14]. Cyclin D1 forms an active complex with two cyclin-dependent kinases, CDK4/CDK6, through which it phosphorylates retinoblastoma protein (Rb) [15]. The inactivating phosphorylation of Rb liberates the E2F-transcription factor, and the transcription of genes responsible for the G1–S transition is enhanced [16]. Cyclin D1 is overexpressed in colon adenomas and tumors [17–19], whereas declining tumor burden by dietary treatment has been shown to decrease also cyclin D1 in the aberrant crypt foci and tumors [20]. There is also some evidence that cyclin D1 in the normal-appearing mucosa responds to dietary treatment and may reflect the adenoma formation [20–23].

We have earlier shown that dietary inulin, polydisperse β(2-1) fructan, promotes tumorigenesis in Min/+ mice [24–26], and also in the present study inulin was used to induce adenoma growth. The aim was to further analyze biochemical changes reflecting adenoma formation but which can be found already in the normal-appearing mucosa of Min/+ mice. The promotion of adenoma growth by inulin was associated with the activation of β-catenin signaling. Also in the wild-type mice the drop in membranous β-catenin was clear after inulin feeding, and, in addition, a subset of crypts had enhanced nuclear β-catenin staining.

2. Materials and methods

2.1. Animals and diets

The Laboratory Animal Ethics Committee of the University of Helsinki approved the study protocol. Wild-type and Min/+ mice (C57BL/6J Min/+), which were originally obtained from The Jackson Laboratories (Bar Harbor, ME), were bred at the Laboratory Animal Center of the University of Helsinki. DNA isolated from the tail was used for PCR assay to genotype mice for wild type or heterozygote for the *Apc* allele [27]. Both genotypes were used in the experiment.

During the suckling time, the pups had free access to pelleted standard rodent laboratory chow (Altromin, Ringsted, Denmark) and tap water. At the age of 5 weeks, the mice were weaned and the starting samples were taken from 11 Min/+ and 10 wild-type mice. Male and female mice were stratified by body mass and sex, and divided randomly into two dietary treatment groups, 10–15 Min/+ mice/group and 8–9 wild-type mice/group. Feeding periods of the Min/+ mice were 3 and 10 weeks, and for the wild-type mice 3 weeks. All the mice were fed ad libitum. The animals were housed in plastic cages, three to five mice together, in a temperature- and humidity-controlled animal facility, with 12-h light/dark cycle. In these conditions, food consumption is not possible to measure, but the welfare of the animals and the development of body weights were ensured regularly.

Table 1
Composition of the experimental diets (g/kg)

Ingredient	Nonfiber control	Inulin
Casein	236.2	212.6
Dextrose	479.0	431.1
Butter	148.9	134.0
Sunflower-seed oil	13.3	12.0
Rapeseed oil	62.2	56.0
Mineral mix (AIN93G)	41.6	37.4
Vitamin mix (AIN93GM)	11.8	10.6
L-Cystine	3.6	3.2
Choline chloride	3.6	3.2
Tertiary butylhydroxyquinone	0.014	0.014
Inulin	–	100

Both diets provided similar amounts of protein, fat, vitamins and minerals on an energy basis (Table 1). The control diet was a modified high-fat AIN93-G diet [28] without any added fiber. The fat content of the diet was similar as in an average Western-type diet so that 40% of the energy came from fat, and the ratio between saturated, monounsaturated and polyunsaturated fatty acids was close to 3:2:1. The inulin diet was similar to the control diet but contained 10% (w/w) inulin (polydisperse β(2-1) fructan, RaftilineHP, Orafti, Tienen, Belgium). The amount of inulin was based on our earlier studies [24,25]. Already 2.5% inulin in the diet can promote adenoma growth, but a higher amount was chosen to be able to detect clearer differences in the molecular biological analysis. The diets were stored at −20°C.

2.2. Evaluation of intestinal adenomas and tissue samples

The mice were sacrificed at 8 or 15 weeks of age by CO_2 inhalation. The intestinal tracts were removed, opened longitudinally and washed with ice-cold 0.9 % NaCl solution. The small intestine was divided into five equal sections; the cecum and colon were kept together. Two observers blinded to the dietary treatment measured the diameter of all adenomas in each section using a dissecting microscope under 67× magnification. Adenomas in each section were cut out, and the normal-appearing mucosa was then gently scraped off with a microscope slide. During the procedure, the samples were kept on ice and only during the adenoma enumeration on room temperature. Because the intestine was divided into several parts, the enumeration of each section was quick and sample degradation minimal. This was ensured by testing the samples after several standing times (data not shown). The smallest adenomas that we were able to score had a diameter of 0.3 mm. In unclear situations, the possible adenomas smaller than that were cut out, but they were not included in the adenoma sample. We have earlier ensured that our mucosa samples are practically free of COX-2, which is expressed

mainly in adenoma tissue. Because of the higher number and size of adenomas at 15 weeks of age, the three most distal parts of the small intestine were pooled together to have enough mucosa from this age group. In all other time points, two parts were pooled together. All samples were frozen in liquid N_2 and stored at −70°C.

2.3. Sample preparation

Nuclear, cytosolic and membranous fractions were isolated individually for all mice. All steps for protein isolation were performed at 4°C. Small intestinal mucosa samples were homogenized in 2 ml of ice-cold homogenization buffer [20 mmol/L Tris–HCl (pH 7.4), 10 mmol/L EGTA, 2 mmol/L EDTA, 250 mmol/L sucrose, 2 μl/ml protease inhibitor cocktail P8340 (Sigma-Aldrich, Steinheim, Germany)]. Homogenates were centrifuged for 10 min at 200×*g* to remove intact cells. Cleared supernatants were centrifuged further for 10 min at 1000×*g*. Supernatants were used for cytosolic and membranous fraction isolations, and pellets for nuclear fraction isolations. The ice-cold homogenization buffer was added to the supernatants so that the final volume was 5 ml. These mixtures were centrifuged for 60 min at 100 000×*g*. Newly formed supernatants were collected and used as cytosolic fractions of mucosal epithelial cells. The pellets were resuspended in 5 ml of ice-cold homogenization buffer containing 2 ml/L Triton X-100, incubated for 30 min and centrifuged for 60 min at 100 000×*g*. The resulting supernatants were used as membranous fractions of mucosal epithelial cells. The pellets formed in the first step of the procedure containing nuclear fractions were resuspended in 1.8 ml of ice-cold homogenization buffer containing 2 ml/L Triton X-100, incubated for 20 min and centrifuged for 10 min at 15 000×*g*. The resulting supernatants were used as nuclear fractions of mucosal epithelial cells. All fractions were concentrated using Amicon Ultra-4 Centrifugal Filter Devices (Millipore, Bedford, MA). The protein concentrations of the samples were measured using a Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA). Equal volumes of the sample and SDS sample buffer were mixed, boiled for 3 min and stored at −70°C. The purity of cellular fractions was controlled by determining the nuclear lamin B (Sc-6216, Santa Cruz Biotechnology, Santa Cruz, CA) levels in the cellular fractions. Both the cytosol and membrane fractions were free of lamin B.

2.4. Immunoblotting

For the immunoblotting analysis of β-catenin or cyclin D1, 20-μg sample proteins were electrophoresed through 10% SDS-PAGE and transferred to nitrocellulose membranes (Hybond ECL membrane, Amersham Pharmacia Biotech). Rat brain or RAW cell homogenate was run along with samples to control interassay variation. For E-cadherin analysis, the amount of sample used was 15 μg protein, and A431 cell line was used as a positive control. SDS-PAGE gels were 6.5% and not 10% as used for β-catenin or cyclin D1. Each sample was analyzed at least in duplicate. If the difference between the results was more than twofold, the sample was analyzed once more. Membranes were blocked overnight at 4°C in Tris-buffered saline with 0.1% Tween (TBS-Tween) containing 3.5% nonfat soy flour for β-catenin or 5% nonfat dry milk for cyclin D1 and E-cadherin. Membranes were incubated 2 h with primary antibodies: anti-β-catenin (Sc-7199, rabbit polyclonal, Santa Cruz Biotechnology), anti-cyclin D1 (33-3500, mouse monoclonal, Zymed, San Francisco, CA), anti-E-cadherin (610182, mouse monoclonal, BD Transduction, San Diego, CA). Protein detection was carried out using secondary antibodies (Santa Cruz Biotechnology), anti-rabbit HPR (Sc-2030), anti-mouse HPR (Sc-2031), anti-mouse HPR (Sc-2005) and an enhanced chemiluminescence (ECL) detection system (Amersham, Arlington Heights, IL). Blots were scanned and analyzed using GS-710 Calibrated Imaging Densitometer and the Quantity One program (Bio-Rad Laboratories). The results are expressed as sample band intensity (optical density of protein band multiplied by band area) divided by rat brain, RAW or A431 band intensity. Equal loading of samples was ensured by incubating blots with β-actin antibody (A5441, Sigma-Aldrich) or lamin antibody (Sc-6216, Santa Cruz). In addition, the widths of the lanes were compared. Blocking peptides, immunoprecipitation, other commercially available antibodies or normal serum was used to ensure detecting the right β-catenin, cyclin D1 and E-cadherin bands.

2.5. Immunohistochemistry

At the age of 8 weeks, five mice were chosen from each experimental group for immunohistochemical analyses. Tissues were fixed in 4% PFA overnight, and serial 5-μm sections of the distal small intestinal mucosa from the wild-type and Min/+ mice were deparaffinized and rehydrated. After quenching endogenous peroxidases in 3% H_2O_2, slides were rinsed in TBS, and an antigen-retrieval step was carried out in microwave oven for 15 min in pH 6.0 citrate buffer. Immunostaining with anti-β-catenin (Transduction Laboratories, Lexington, KY) at dilution 1:200, anti-E-cadherin (Transduction Laboratories) at dilution 1:1000, anti-cyclin D1 (Zymed, San Francisco, CA) at dilution 1:500, and counterstaining with Mayer's hemalaun (Merck) were performed using a Homo-mouse Poly-HRP IHC Detection Kit (KDM-7DAB, ImmunoVision Technologies, Brisbane, CA). Negative control tissues were prepared in the same manner, except the primary antibody was replaced with negative control for mouse IgG_{2a} Ab-1 (NeoMarkers, Lab Vision, Fremont, CA). Staining for β-catenin, E-cadherin and cyclin D1 proteins was scored on the bases of distributions and relative staining intensities by two observers blinded to the dietary treatment.

2.6. Statistical analyses

Results are expressed as median (range) and shown as box plots. The differences between the groups were

analyzed by Mann–Whitney *U* test. Spearman correlation was used for correlation analysis (StatView, version 5.0.1, SAS Institute, Cary, NC). Significant difference was considered as $P<.05$.

3. Results

3.1. Weight gain and the adenoma growth

Generally, mice grew well and the final body weights were similar in both dietary groups. The male and female mice did not differ at any of the time points, and therefore the data from the male and female mice are pooled in the results. Most of the adenomas developed into the distal part of the small intestine and the average diameter of adenomas was significantly greater in the inulin group at both time points (Fig. 1A).

3.2. Cyclin D1

The promotion of adenoma growth in the inulin group was associated with cyclin D1 accumulation in the cytosol of the normal-appearing mucosa (Fig. 1B). At week 15, the amount of cyclin D1 was increased by 1.5-fold in the inulin group when compared to the controls [1.22 (2.30) inulin vs. 0.71 (2.67) control, $P=.003$]. We were not able to obtain cyclin D1 signals from the nuclear fractions by Western blotting although we tested several commercially available antibodies. However, in the cytosol fractions, the signals were very clear (Fig. 2). We were able to perform immunohistochemistry from 8-week-old mice (Fig. 3A), and it confirmed the cellular localization seen by Western blotting. Cyclin D1 in the normal-appearing mucosa was cytoplasmic, whereas nuclear staining was very rare. No statistical difference between the dietary groups could be

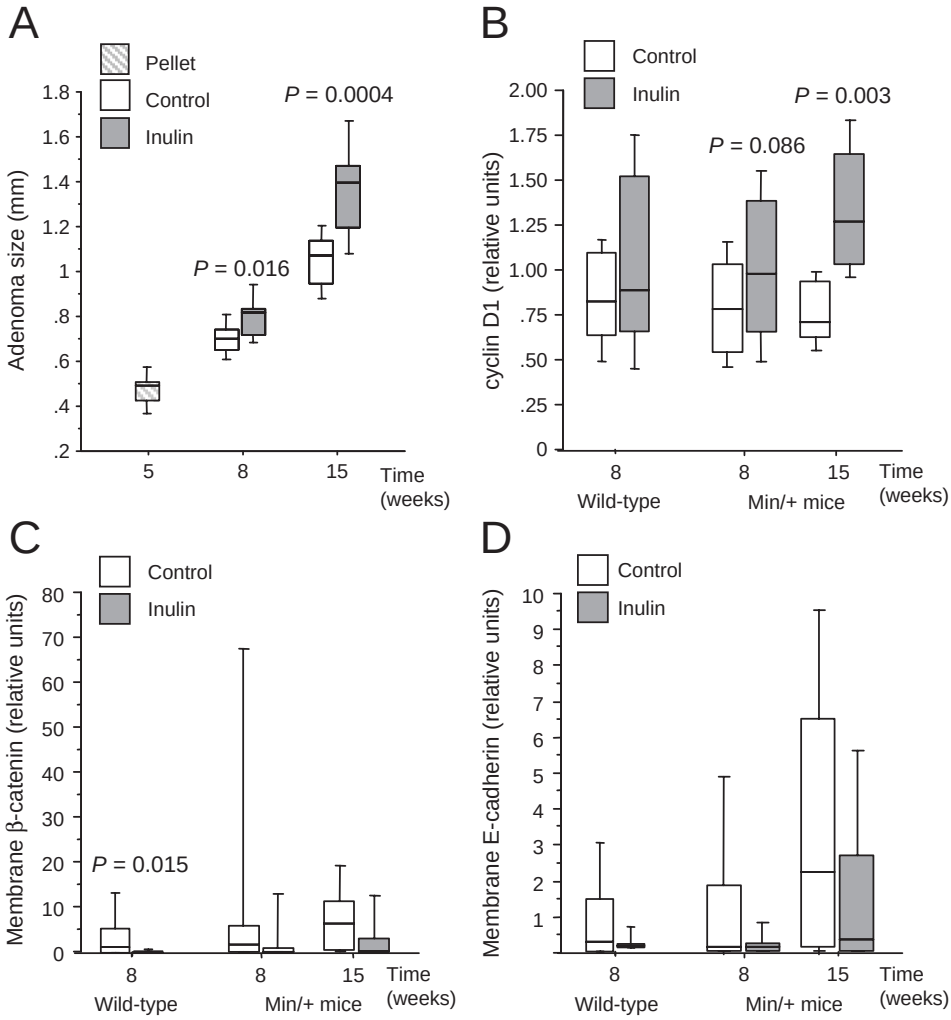

Fig. 1. Inulin increased the adenoma size and amount of cyclin D1 together with the decrease in the cell adhesion proteins. (A) Adenoma size (diameter, mm) in the distal small intestine of Min/+ mice expressed as a box plot. (B) Cyclin D1 levels (median) in the cytosol, (C) β-catenin levels (median) in the membranes, (D) E-cadherin levels (median) in the membranes of enterocytes. The Min/+ mice ($n=10–15$ per group) and the wild-type mice ($n=8–9$ per group) were fed either a nonfiber control diet or an inulin diet (10%) from the age of 5 weeks until the age of 8 or 15 weeks. Until the age of 5 weeks, the mice were fed a pelleted, standard rodent chow. Protein levels were measured by immunoblotting and results are represented as relative intensities. Probability (P) in comparison to control mice of the same age by Mann–Whitney *U* test.

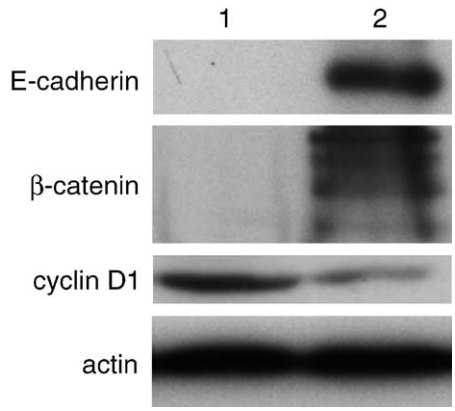

Fig. 2. Representative immunoblot of membranous E-cadherin, membranous β-catenin and cytosolic cyclin D1 in the normal-appearing mucosa of the Min/+ mice fed either an inulin diet (1) or a nonfiber control diet (2) at the age of 15 weeks.

detected by immunohistochemistry at the age of 8 weeks, which was in line with the Western blotting results at the same age. Different studies have controversial results about cellular localization of cyclin D1. In the late 1990s, Arber et al. [18] and Zhang et al. [19] determined cyclin D1 from the intestine and they did not get any nuclear staining from the normal-appearing mucosa. Later studies have confirmed that cyclin D1 staining may be weak [17] and cyclin D1 in the mucosa is mainly cytoplasmic [18,21].

3.3. β-Catenin

To determine whether dietary inulin could change the amount of cellular β-catenin or its localization between subcellular fractions in a way that might promote cyclin D1 production, β-catenin was determined by Western blotting from membranous (Fig. 2), cytosolic and nuclear fractions, and also by immunohistochemistry (Fig. 3B and C). As in our earlier Western blot analyses [25], the Min/+ mice mucosa contained several bands specific for β-catenin. Accordingly, we used the sum of all β-catenin bands. Although the results obtained by the Western blot analyses did not reach the statistical significance between the treatment groups, the inulin group showed stronger β-catenin staining by immunohistochemistry. On the scale from 1 to 3, the inulin group scored 2.8 and the control group 2.2 at the age of 8 weeks. Dietary treatment also distributed β-catenin differently to cell compartments. In the control group, β-catenin stained in the cytosol and strongly in the membranes, but the inulin diet seemed to release β-catenin from the membranes and cause its translocation to the nucleus. Nuclear staining in the crypt area was strong in four of five inulin mice, but it was not seen in the control mice.

3.4. E-Cadherin

To further explore the impact of reduced membrane β-catenin (Figs. 1C, 2, 3B and C) on possible impairment of cell adhesion, we analyzed the amount of E-cadherin from the membrane fraction of the intestinal mucosa of the Min/+ mice (Figs. 2, 3F and G). The correlation between E-cadherin and β-catenin in the membranes was strong (Western blotting, week 15, $r = .9$, $P < .0001$), and immunohistochemical staining confirmed that inulin feeding decreased the amount of E-cadherin in the Min/+ mice mucosa. On the scale from 1 to 3 the inulin group scored 1.2 and the control group 1.9 for E-cadherin staining at the age of 8 weeks. Surprisingly, in the Western blot analysis the large number of observations was under the detection limit, and because of that the trend for reduced amount of E-cadherin in membranes could not be analyzed statistically (Fig. 1D). E-Cadherin was absent in 60% (12/20) of observations in the inulin group and in 46% (12/26) in the control group. We were not able to explain this disappearance, since we used the same amount of protein in all analysis, and loading markers gave similar results for all

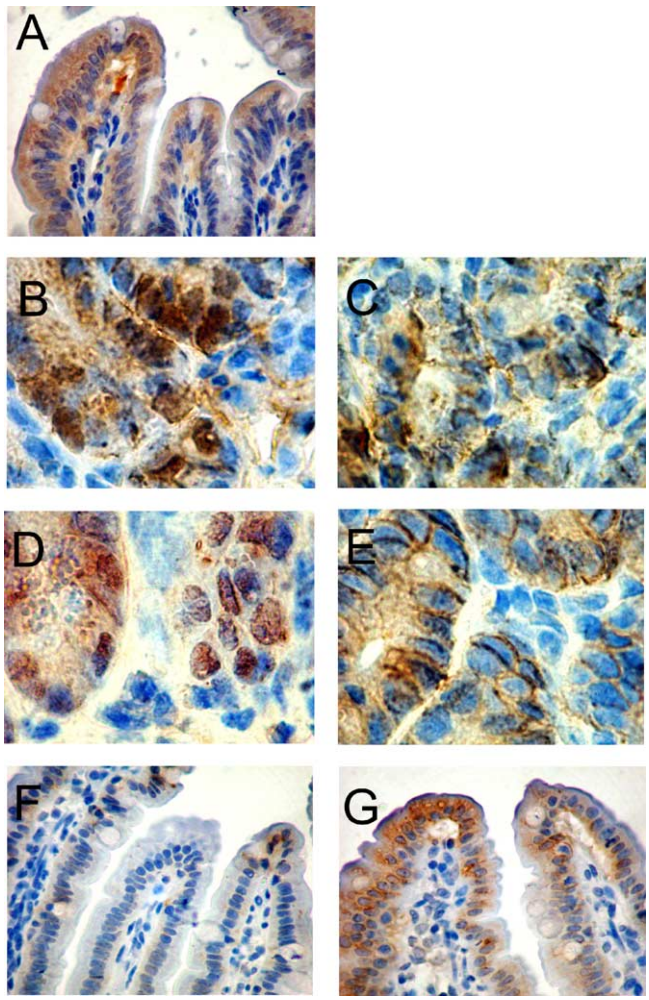

Fig. 3. Immunohistochemical staining of cyclin D1 (A), β-catenin (B, C) and E-cadherin (F, G) in the normal-appearing mucosa of the Min/+ mice, and β-catenin (D, E) in the mucosa of the wild-type mice (magnification ×400 and ×1000). The expression of cyclin D1 was similar in both dietary groups at the age of 8 weeks, and here is presented the inulin group (A). After the control diet, β-catenin was mainly membranous (C, E), whereas inulin diet induced its translocation to the nucleus (B, D). The staining of E-cadherin was very weak in the inulin group (F) compared to the control group (G).

samples. To clarify the zero findings, we analyzed the data again without mice lacking the signals for membrane proteins. Still the adenoma data and increased amount of cyclin D1 in the inulin group stayed constant and confirmed the results. The zero findings in Western blotting do not mean that analyzed proteins are totally missing. When the large section of intestinal mucosa is homogenized, immunoblotting gives an insight into the overall situation.

3.5. Wild-type mice

To determine whether the changes caused by dietary inulin were common for intestinal mucosa or just specific for the Min/+ mice, we analyzed the same parameters from the wild-type littermates that had gone through the same feeding procedure as the Min/+ mice from the age of 5 weeks to the age of 8 weeks. Generally, when the Min/+ mice and the wild-type littermates were compared at 5 weeks of age, the wild-type mice had more β-catenin (P=.017) and E-cadherin (P=.008) than the Min/+ mice in the membranes. The wild-type mice had also more cyclin D1 (P=.002) than the Min/+ mice. Surprisingly, in the wild-type mice, inulin feeding for 3 weeks already caused a reduction in membranous β-catenin levels (Fig. 1C) [1.04 (14.90) control vs. 0.06 (0.81) inulin, P=.015]. Immunohistochemical analyses showed that in the inulin group a subset of crypts had nuclear β-catenin staining (Fig. 3D and E).

4. Discussion

This study showed that the fructo-oligosaccharide inulin increased the size of the small intestinal adenomas of Min/+ mice. The growth promotion was accompanied with accumulation of cytosolic and nuclear β-catenin, and increased amount of cytosolic cyclin D1 in the normal-appearing mucosa. Furthermore, inulin feeding reduced the levels of β-catenin and E-cadherin in the mucosal membranes. In the wild-type mice the drop in membranous β-catenin was clear, and, in addition, a subset of crypts also had enhanced nuclear β-catenin staining.

Increasing levels of nuclear β-catenin and the activation of β-catenin/Tcf pathway are the primary transforming events in colon cancer as the target genes of β-catenin control whether cells in the intestinal epithelium proliferate or differentiate [5,29]. E-Cadherin can influence the availability of β-catenin by competing with other binding partners of β-catenin, and therefore β-catenin is suggested to have nuclear activity only when it is free of E-cadherin [30,31].

Histologically, normal-appearing enterocytes in the small intestine of Min/+ mice show a 25% reduction in migration rate compared to their wild-type littermates [32]. Min/+ mice have impaired enterocyte proliferation and apoptosis, as well as defects in cell–cell adhesion reflecting the reduced association between E-cadherin and β-catenin [33]. Our results are in accordance with these observations since at 5 weeks of age the Min/+ mice had less membranous E-cadherin and β-catenin, and cytosolic cyclin D1 in the mucosa than their wild-type littermates. It may be speculated that as the enterocyte migration is reduced even a relatively small shift in the proliferation can cause crowding in the crypt area. In these situations cells may not stay anymore in strict order as also cell–cell contacts are weakened.

The cell-cycle regulator cyclin D1 is involved in the development of colon cancer [17–19,34]. Cyclin D1 may not be essential for intestinal tumorigenesis, but it acts as a modifier of disease severity [35], and cyclin D1 deficiency reduces intestinal polyp formation [36]. The notion that increased epithelial cell proliferation in the normal-appearing colonic mucosa means a higher risk for neoplasms [37] also supports our results. Cyclin D1 itself does not necessarily cause the cell cycle progression, because small molecules such as p21 and p27 can antagonize cyclin D1 function. However, our previous results did not give any sign that inulin feeding would increase p53 signaling in the mucosa of Min/+ mice [25].

In our earlier study, inulin feeding decreased the amount of proliferating cell nuclear antigen (PCNA) in the normal-appearing mucosa of Min/+ mice which was thought to be a result of impaired migration [25]. However, cyclin D1 and PCNA may not have a simple correlation. It has been suggested that cyclin D1 overexpression is not a mere consequence of cellular proliferative activity but rather represents a true difference between the normal and tumorous states [38]. Cyclin D1 has also been suggested to be involved in signaling pathways regulating cellular differentiation and migration [35,39].

Some studies have shown that inulin may reduce chemically induced colon cancer [40,41], which is mainly demonstrated by the suppression of aberrant crypt foci formation. The actual tumors have been investigated only in a few experiments [40,42]. The controversy between these and our findings may be a result of different colon cancer models used, as the mutational background varies. Azomethane, the most commonly used chemical carcinogen, causes β-catenin and K-ras mutations [43] that are usually found in the later stages of adenoma–carcinoma sequence. The *APC* mutation is the earliest change found in inherited FAP as well as in most sporadic colon cancers. In addition, adenomas are a suitable target for treatment intervention because of their phenotypic and genotypic similarities and evolutionary proximity to invasive cancer [44]. Therefore, the situation seen in Min/+ mice may be relevant also in humans.

In the earlier study, we compared our fiber-free control diet with low-fat AIN93-G diet that has cellulose as a fiber source [24]. Adenoma numbers in these diets did not differ, and, therefore, we believe that inulin has similar effects regardless of the basic diet. It is speculated that inulin does not have any effect on the small intestinal mucosa in the absence of colonic fermentation, but the data obtained in the Min/+ mice contradict this assumption. Our controversial results against the overwhelming healthfulness of inulin are not unique, since inulin has also been shown to impair

mucosal barrier by the rapid production of fermentation metabolites [45].

In conclusion, this study provides evidence that dietary fructo-oligosaccharide inulin can, at least in high-fat background, activate Wnt signaling: E-cadherin–β-catenin complexes are released from cell membranes, β-catenin accumulates in cytosol and nucleus, and the amount on its target protein cyclin D1 is increased. This is not specific only for Min/+ mice since the changes were also seen in wild-type mice. The changes in the cellular balance are not deleterious in the wild-type mice, and epithelial cells can preserve their function. However, in the Min/+ mice, β-catenin signaling seems to be connected to increased amounts of cellular cyclin D1 and to the promotion of intestinal adenoma growth.

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