Eur J Nutr (2008) 47:115–122
DOI 10.1007/s00394-008-0704-0

ORIGINAL CONTRIBUTION

Johanna Rajakangas
Marjo Misikangas
Essi Päivärinta
Marja Mutanen

Chemoprevention by white currant is mediated by the reduction of nuclear β-catenin and NF-κB levels in Min mice adenomas

Received: 3 August 2007
Accepted: 12 March 2008
Published online: 3 April 2008

J. Rajakangas and M. Misikangas contributed equally to this work.

J. Rajakangas (✉) · M. Misikangas
E. Päivärinta · M. Mutanen
Dept. of Applied Chemistry
and Microbiology (Nutrition)
University of Helsinki
P.O. Box 66
00014 Helsinki, Finland
Tel.: +358-9/1915-8274
Fax: +358-9/1915-8269
E-Mail: johanna.rajakangas@helsinki.fi

Abstract *Background* Berries are a good natural source of phenolic compounds and many berries or their compounds have been shown to be chemopreventive. White currant is an interesting berry, as it contains low levels of dominant berry phenolics such as ellagic acid, anthocyanins and other flavonoids. *Aims of the study* To study if white currant is chemopreventive in an experimental model for intestinal tumorigenesis and further study the effects on β-catenin and NF-κB signaling pathways. *Methods* Multiple intestinal neoplasia (Min) mice were fed an AIN-93G based control diet or a diet containing 10% freeze dried white currant (*Ribes x pallidum*) for 10 weeks. Cell signaling parameters were analysed from intestinal adenomas and surrounding mucosa by Western blotting and immunohistochemistry. *Results* The white currant diet reduced the number of adenomas from 81 (min–max 47–114) to 51 (36–84) in the total small intestine of Min mice ($P < 0.02$). Most of the adenomas develop in the distal part of the small intestine, and in this area white currant reduced the number from 49 to 29.5 ($P < 0.01$) and also the size of the adenomas from 0.88 mm to 0.70 mm ($P < 0.02$). In the colon white currant increased the number of adenomas (0.3 ± 0.6 vs. 0.8 ± 0.6, mean ± SD, $P < 0.05$), but did not affect the size. White currant reduced nuclear β-catenin and NF-κB protein levels in the adenomas ($P < 0.05$ and $P < 0.02$, respectively). They were correlated with the size of adenomas ($P < 0.01$). *Conclusions* This study shows that white currant is effective in preventing cancer initiation and progression in the Min mouse. Whether the positive effects are due to its special phenolic composition needs to be studied in more detail.

Key words white currant – colon cancer – Min mouse – β-catenin – NF-κB

Introduction

Diet is an important contributor to the development of colon cancer [46] and diet can either promote or prevent cancer formation and progression. It is widely accepted that consumption of a diet high in plant-based foods is associated with a decreased risk of many types of cancer. Berries contain promising bioactive compounds that may inhibit the cells becoming malignant [11, 18] since many of the phenolic compounds such as anthocyanins [22, 26, 40, 48, 50], proanthocyanidins [13, 40], phenolic acids [48] and ellagitannins [1] are found to have chemopreventive effects in colon cancer cells in vitro. We

have earlier shown in vivo that berries rich in these compounds; bilberry, lingonberry, and cloudberry [21, 27, 35] are anticarcinogenic in multiple intestinal neoplasia (Min/+) mice [14]. Also other studies confirm the chemoprevention of berries in vivo; black raspberry (*Rubus occidentalis)*, anthocyanin-rich extracts of bilberry (*Vaccinum myrtillus*), chokeberry (*Aronia melanocarpa)*, and grape (*Vitis vinifera*) as well as an anthocyanin mixture from bilberry have inhibited cancer formation in animal models [10, 17, 29].

The chemoprevention studies so far have mainly concentrated on the phenolics of the berries. In this study, we used a colorless berry—white currant (*Ribes x pallidum*)—that contains only very low levels of dominant berry phenolics such as ellagic acid, anthocyanins and other flavonoids [21, 34, 35]. Instead, it contains some proanthocyanidins, as well as hydroxybenzoic acid and hydroxycinnamic acid derivatives such as caffeic acid and ferulic acid.

We investigated how white currant affects adenoma formation and cell signaling in Min mice, a widely used model of colon cancer that due to a heterozygous mutation in the adenomatous polyposis coli (*Apc)* gene develops dozens of intestinal tumors [33, 41]. Also the effect of the white currant diet on two important signaling pathways in cancer biology, the Wnt and nuclear factor -κB (NF-κB) pathways, was analyzed. Both Wnt and NF-κB pathways are currently being studied as cancer drug targets [12, 25, 30]. For example, a wide variety of anti-inflammatory drugs seem to elucidate their chemopreventive effects by inhibition of these two pathways [5, 28, 47, 49]. As reviewed by Gillmore and Herscovitch, there are over 700 known inhibitors of NF-κB, many of which are known cancer drugs, but they also include a large variety of different natural compounds [15].

By understanding how diet or a specific dietary constituent affects these signaling pathways it is possible to get valuable information on how colon cancer can be prevented by dietary means. The aim of this study was therefore to see if the results obtained earlier with the berries rich in anthocyanins or other flavonoids could be reproduced by a berry that practically lacks these compounds [21, 34, 35]. In addition, the mechanism whereby white currant affects cancer formation was studied by measuring β-catenin, the main protein of the Wnt-pathway, and NF-κB levels from the adenomas and surrounding mucosa in Min mice intestine after white currant feeding.

Materials and methods

Animals

The Laboratory Animal Ethics Committee of the University of Helsinki, Finland, approved the study protocol. *Min*/+ mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and male and female C57BL/6J *Min*/+ mice were bred and treated at the Experimental Animal Unit of the University of Helsinki. At the age of five weeks the animals were randomly divided in to two groups and the diet of the pups was changed from a standard rodent laboratory chow (Altromin, Ringsted, Denmark) to the experimental diets: control (n = 11, 5 male and 6 female) and white currant (n = 12, 6 male and 6 female). During the 10-weeks feeding period the mice had free access to food and water.

Diets

The control diet was a high-fat AIN-93G based diet [39], providing 41% of energy from fat, 39% from carbohydrates and 19% from protein (Table 1). The white currant diet contained 10% (w/w) freeze-dried white currant (*Ribes x pallidum*) (Marja Carelia, Kiihtelysvaara, Finland) and provided similar amounts of fat, carbohydrates and protein as the control diet on an energy basis. This means that when eating the same amount of energy, the diets provided similar amounts of fat, carbohydrate, protein, as well as other components of the diets, except for the ones provided by white currant. In addition to non-nutrients from the white currant, it also provided some fiber (19 g/kg diet), which was absent from the fiber-free control diet. The level of white currant calculated as fresh berries was 475 g/kg diet. The fat content of the diets was adjusted to a Western type diet, containing saturated, monounsaturated and polyunsaturated fatty acids in the ratio 3:2:1.

Tumor count and sample preparation

After the feeding period, the mice were killed by CO_2 inhalation and the small intestine, cecum, and colon were removed, opened along the longitudinal axis,

Table 1 Composition of experimental diets

Ingredient (g/kg)	Control	White currant
Casein	236.2	217
Dextrose	479.0	414.3
Butter	148.9	141.7
Sunflower-seed oil	13.3	10.4
Rapeseed oil	62.2	59.2
Mineral mix (AIN93G)	41.6	39.6
Vitamin mix (AIN93GM)	11.8	11.2
L-cystine	3.6	3.4
Choline chloride	3.6	3.4
Tertiary butylhydroxyquinone	0.014	0.013
Freeze-dried white currant	–	100

and rinsed with ice-cold saline. The small intestine was divided into five equal sections. The two distal parts of the small intestine are referred to as the distal small intestine and the two parts in the middle as the middle small intestine and the most proximal part as the duodenum. Two observers blinded to the dietary treatment measured the diameter of all adenomas in each section using a dissecting microscope under 67× magnification. Adenomas were excised and pooled together depending on the section. The normal appearing mucosa was gently scraped off with two microscope slides. During the procedure the samples were kept on ice. The area of the adenomas was calculated based on the diameter and number of adenomas. The smallest detectable adenomas had diameter of 0.3 mm. In unclear situations, the possible adenomas smaller than that were removed but they were not included in the adenoma sample. All samples were frozen in liquid N_2 and stored at −70°C.

Western blotting

Sample preparation and Western blotting has been described in more detail in our previous study [31]. Briefly, normal appearing mucosa and adenomas in the distal small intestine were further fractionated to nuclear, cytosolic, and membranous pools for each mouse. Proteins were separated on a 10% SDS gel and transferred to a nitrocellulose membrane and blocked over night in 3.5% soy-flour (β-catenin) or 5% non-fat milk in TBS-Tween (0.01%) (p65, cyclin D1, p53, actin, lamin). Membranes were incubated in primary antibody for 2 hours, washed and incubated in HRP-conjugated secondary antibody. Bands were visualized using the Enhanced Chemiluminescent system (Amersham Pharmacia Biotech, Little Chalfont, UK). For Western blotting analyses the following primary antibodies were used: anti-β-catenin (Sc-7199, Santa Cruz Biotechology, Santa Cruz, CA, USA), anti-p65 (Sc-372, Santa Cruz Biotechology), anti-cyclin D1 (RM-9104, NeoMarkers, Lab Vision, Fremont, CA, USA) or anti-p53 (RB-9006, NeoMarkers). To all gels, duplicates of an internal standard were added. The bands were analyzed using the GS-710 Calibrated Imaging Densitometer and the Quantity One—program (Bio Rad, Hercules, CA, USA). The bands of the samples were divided with the average of the internal standard to correct for differences between gels and runs. Equal loading of samples was ensured by incubating blots with β-actin (A5441, Sigma-Aldrich) or lamin antibody (Sc-6216, Santa Cruz Biotechology). Blocking peptides, immunoprecipitation, other commercially available antibodies or normal serum were used to ensure detecting right bands (data not shown).

Immunohistochemistry

Tissue samples were taken from the proximal part of the ileum from all mice, so that most samples contained the adenoma in addition to the normal appearing mucosa. Immunostaining with anti-β-catenin (Transduction Laboratories, Lexington, KY, USA) and anti-cyclin D1 (NeoMarkers) was performed using PowerVision Homo-mouse IHC Detection Kit (KDM-7DAB, ImmunoVision Technologies Company, Brisbane, CA, USA) or UltraVision Anti-Rabbit Detection System (TR-015-HD, Lab Vision UK, Suffolk, UK) [6]. Two observers blind to the dietary treatment evaluated the IHC staining intensities with a scale ranging between 0 (no staining) and 3 (very strong staining).

Statistical analyses

The results are expressed as the median (min–max), as the non-parametrical distribution requires non-parametrical analysis that compares medians instead of means. The result of colon adenomas and immunohistological staining intensities of cyclin D1 is presented as mean ± SD. The data were analyzed statistically using the nonparametric Mann–Whitney *U*-test for independent samples and Spearman analysis for correlation (SPSS, v. 10.0, Chicago, IL, USA or StatView, version 5.0.1, SAS Institute Inc., Cary, NC, USA). Differences were considered significant when $P < 0.05$.

Results

White currant reduces adenoma number and affects adenoma size in the Min mouse

The animals grew well and the final body weights were the same in both groups (Fig. 1) indicating that the groups consumed similar amounts of the diets and that there were no adverse side-effects of white currant. Except for smaller body weight, no difference in adenoma number or size or any analyzed protein parameter was observed between females and males. Compared to the control diet, the white currant diet reduced the number of adenomas from 81 (47–114) to 51 (36–84) in the total small intestine of Min mice ($P < 0.02$), but had no significant effect on size (Table 2). Most of the adenomas develop in the distal part of the small intestine, and in this area white currant reduced the number from 49 to 29.5 ($P < 0.01$) and also the size of the adenomas from 0.88 mm to 0.70 mm ($P < 0.02$) (Fig. 2). In addition, in the distal small intestine significant difference in size distribution of small adenomas ($P < 0.02$) was also found (Table 2). When comparing the area of adenomatous

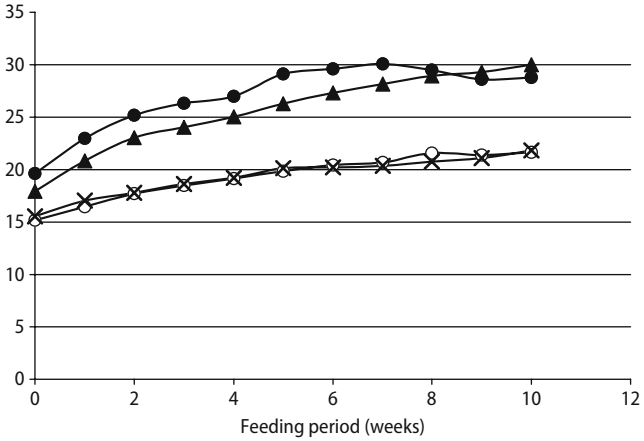

Fig. 1 Weight gain of the Min mice that were fed either a control diet or a diet containing 10% freeze-dried white currant for 10 weeks. (–*filled circle*–), Control, male; (–*open circle*–), control, female; (–*filled triangle*–), white currant, male; (–*cross symbol*–), white currant, female

tissue in the total and distal small intestine, white currant reduced it by 35% ($P < 0.05$) and 65% ($P < 0.01$) compared to the control, respectively. White currant reduced the number of adenomas in the duodenum from 7 to 3 ($P < 0.01$), but increased the size from 1.18 to 1.72 mm ($P < 0.02$). In the colon, white currant, on the other hand, increased the number of adenomas (0.3 ± 0.6 vs. 0.8 ± 0.6, mean ± SD, $P < 0.05$), but did not affect the size.

White currant affects β-catenin signaling in the adenomas

In the adenomas, white currant reduced the protein level of nuclear β-catenin ($P < 0.05$) (Figs. 3, 4), measured by Western blotting. Due to lack of sample material the expression of cyclin D1 could not be analyzed by Western blotting but immunohistochemical staining intensities showed a reduced trend for cyclin D1 in the adenomas of white currant fed mice (2.2 ± 0.8 vs. 1.6 ± 0.5, mean ± SD) (Fig. 4). Due to the low number of samples ($n = 5$/group) no statistical analysis was performed. The attenuated β-catenin signalling seems to be one of the reasons for smaller adenoma size in the white currant group as nuclear β-catenin strongly correlated with adenoma size ($r = 0.594$, $P < 0.01$) (Fig. 3b). White currant feeding did not affect the levels of β-catenin, and cyclin D1 in different cell compartments in the normal appearing mucosa.

NF-κB levels are reduced in the adenomas after white currant intervention

NF-κB was measured as the p65 (RelA) subunit in nuclear preparations. Nuclear p65 protein levels in the adenoma tissue were lower in the white currant group compared to the control group ($P < 0.02$), measured by Western blotting (Fig. 3), but no difference was found in the mucosa. Similarly with the nuclear β-catenin, also nuclear p65 in the adenoma correlated with adenoma size ($r = 0.682$, $P < 0.01$). The level of p53, a target of NF-κB and a key apoptotic protein, was slightly increased in the mucosa of the white currant group [0.59 (0.37–2.77) vs. 0.50 (0.25–0.78), $P < 0.1$]. In the adenoma, no difference in p53 was found.

Table 2 The number of adenomas in the small intestine and colon in Min mice fed with control non-fibre and 10% white currant diets for 10 weeks

	Control	White currant
Number of adenomas		
Total small intestine	81 (47, 114)	51 (36, 84)*
Distal small intestine	49 (26, 64)	29.5 (15, 51)**
Middle small intestine	21 (12, 42)	20 (13, 32)
Duodenum	7 (3, 11)	3 (1, 10)**
Colon (Mean ± SD)	0.3 ± 0.6	0.8 ± 0.6***
Diameter of adenomas (mm)		
Total small intestine	0.99 (0.71, 1.23)	0.95 (0.79, 1.19)
Distal small intestine	0.88 (0.58, 1.21)	0.70 (0.58, 0.91)*
Middle small intestine	1.21 (0.80, 1.45)	1.24 (0.80, 1.85)
Duodenum	1.18 (0.70, 1.56)	1.72 (0.55, 3.00)*
Colon (Mean ± SD)	3.91 ± 0.12	3.49 ± 0.68
Total area of adenomas (mm^3)		
Total small intestine	57 (24, 100)	37 (24, 82)***
Distal small intestine	32 (11, 62)	11 (6, 33)**
Middle small intestine	29 (9, 35)	24 (12, 37)
Duodenum	5 (1, 15)	9 (0.5, 16)
Colon (Mean ± SD)	18 ± 7	11 ± 5
Size distribution of adenomas[a]		
Total small intestine		
Small (%)	55 (38, 83)	65 (42, 77)
Large (%)	16 (6, 28)	16 (9, 23)
Distal small intestine		
Small (%)	60 (31, 93)	81 (58, 92)*
Large (%)	5 (0, 28)	0 (0, 15)
Middle small intestine		
Small (%)	50 (35, 72)	46 (15, 71)
Large (%)	32 (6, 40)	28 (15, 69)
Duodenum		
Small (%)	43 (25, 100)	29 (0, 100)
Large (%)	25 (0, 50)	50 (0, 100)***

Values for the small intestine are median with minimum and maximum values in parentheses Values for the colon are mean ± SD
* Different from control, $P \leq 0.02$; ** $P \leq 0.01$; *** $P \leq 0.05$
[a] Adenomas were categorized as small (diameter ≤ 0.9 mm), medium (1.0–1.5 mm; data not shown) and large (≥1.6 mm) adenomas and proportion of each size category was calculated as per cent of all adenomas

Discussion

Our results show that white currant both prevented the formation of new adenomas and reduced the growth of adenomas in the distal small intestine of Min mice, where most adenomas develop. In the duodenum, the adenomas grew larger in the white currant diet, but since the number of adenomas

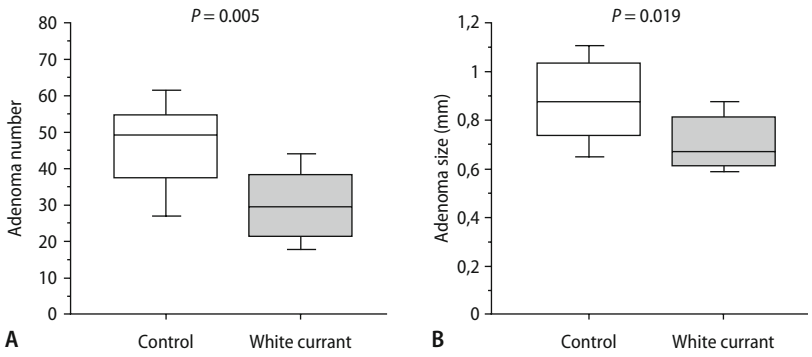

Fig. 2 Min mice were fed either a control diet or a diet containing 10% freeze-dried white currant for 10 weeks. **a** The number of adenomas in the distal small intestine, where most adenomas develop, was reduced in Min mice after white currant feeding. **b** The size (mm) of the adenomas was decreased in the white currant group. Results are presented as *box-plots*, where the *box* represents the interquartile range, which contains 50% of values. The *whiskers* extend from the box to the maximum and minimum values. The median is indicated by a *line across the box*

decreased, the total area was not different between the diets. A large amount of different bioactive compounds entered the duodenum after white currant feeding and may explain the result. The amount of bioactive compounds was probably much less in the distal small intestine suggesting that also a lower amount of white currant could be protective. A dose-response study is needed to find at what level chemoprevention can be achieved. In the colon, even though the number of adenomas was increased by white currant, the area of adenomatous tissue was not increased. Only a fraction of mice developed adenomas in the colon, so this result needs to be confirmed with larger group size.

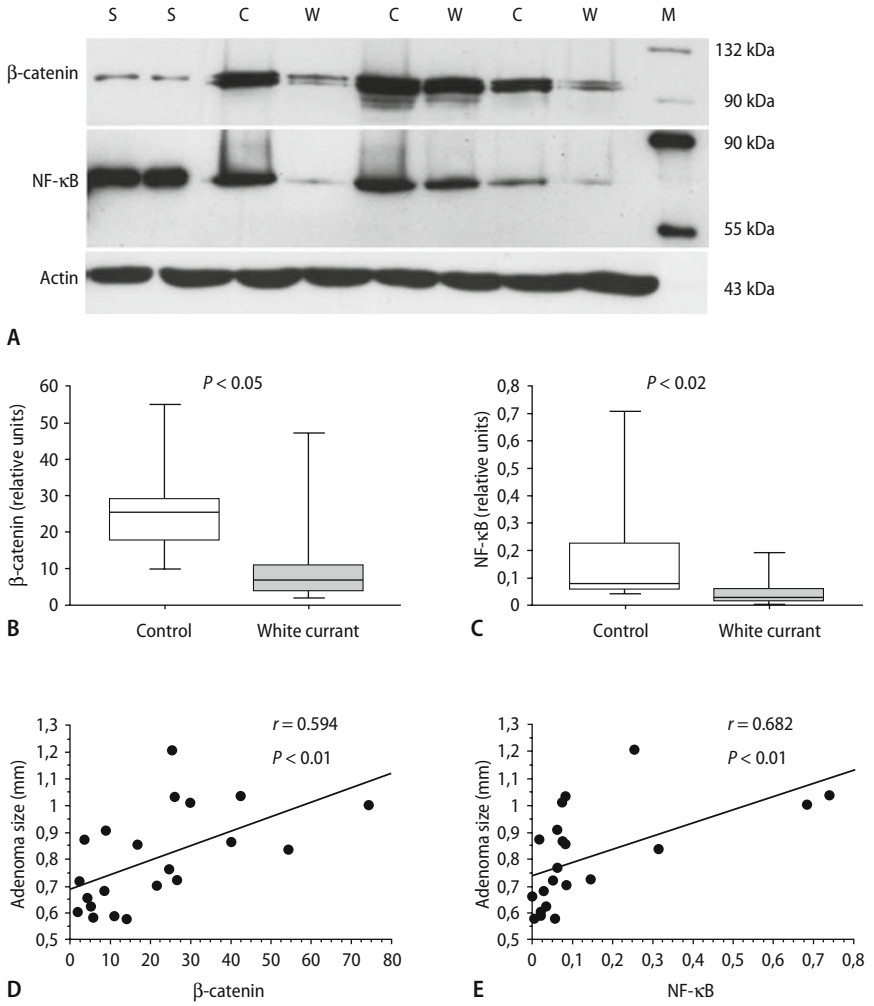

Fig. 3 Feeding white currant (10% w/w) to Min mice significantly decreased protein levels of nuclear β-catenin and the NF-κB subunit p65 in the adenomas of Min mice. **a** Representative Western-blots for β-catenin and NF-κB. *S* internal standard, *C* control diet, *W* white currant diet. **b** *Box-plot* showing the level of nuclear β-catenin as relative units from all ($n = 11$) animals. **c** *Box-plot* showing the level of nuclear NF-κB from all ($n = 11$) animals. **d** A positive correlation was found between nuclear β-catenin and the size of the adenomas. **e** A positive correlation was found between nuclear NF-κB and the size of the adenomas

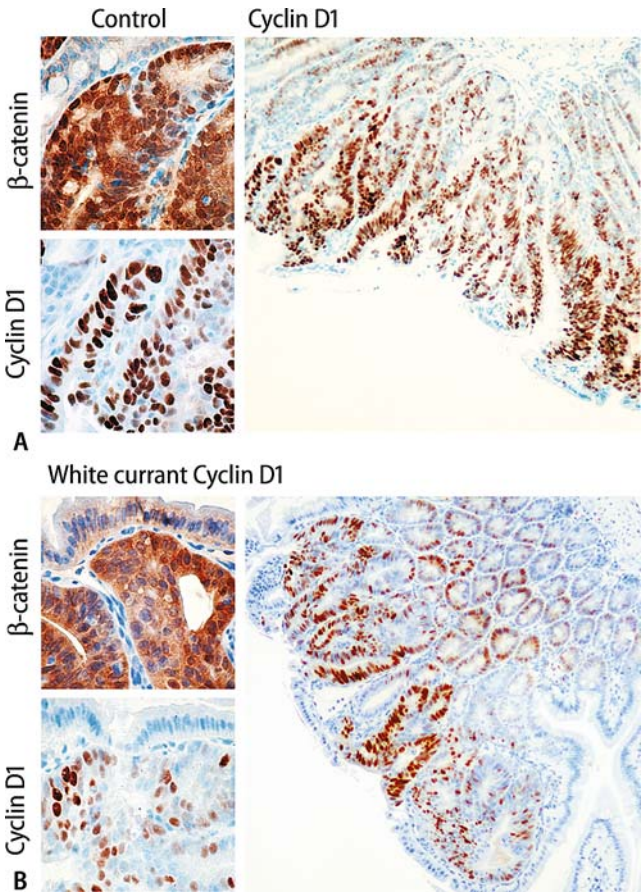

Fig. 4 Representative immunohistochemical staining of β-catenin and cyclin D1 in the adenomas of control (**a**) or white currant -fed mice (**b**). White currant caused reduced nuclear staining of β-catenin and cyclin D1 in the adenomas of Min mice. Magnification 200× in the large picture and 400× in the small pictures. Positive cells show brown staining

White currant (*Ribes x pallidum*) is a colorless berry originating in a mutation in the red currant. White currant, however, has a phenolic profile that differs considerably from red currant; the lack of anthocyanins in white currant is associated with increased levels of phenolic acids [35]. There is however, only a limited set of compounds that have been analyzed from white currant [34, 35] and further investigation on the possible active compounds of this berry is warranted. So far, most studies on berries have focused on the specific action of different phenolic compounds. However, some berries or berry extracts have demonstrated stronger chemopreventive effects than the purified compounds alone [1]. The effects seen in this study may therefore rather be a result of a mixture of compounds acting in synergy than an effect of a single active substance. In fact, we have previously shown that cloudberry is chemopreventive in the Min mouse [14], but cloudberry pulp or seeds alone were ineffective, as was ellagic acid, the main phenolic compound in cloudberry unable to affect tumor formation in the Min mouse [38]. Intake of other nutrients from the freeze-dried white currant hardly can explain the results, either. The amount of fiber in the white currant diet was very small when compared to the earlier studies exploring the effects of fiber on colon tumorigenesis. Effect of white currant on the intake of vitamins and minerals was negligible. For example, the proportions of calcium and folate from the white currant were only 4 and 3% from the total intake of these nutrients, respectively.

White currant inhibited the nuclear accumulation of both β-catenin and NF-κB, indicating that the action of Wnt- and NF-κB pathways was inhibited. The Wnt pathway is disturbed early during colon cancer formation; mutation of the *APC* gene, which is the initiating step in cancer formation, causes inability to degrade cellular β-catenin [9]. The accumulation of β-catenin in the nucleus leads to the transcription of β-catenin target genes related to tumor progression and malignant transformation. The amount of nuclear β-catenin increases in the course of tumor progression and it correlates with metastasis and poor prognosis [16, 20, 42, 44]. The NF-κB pathway is activated by various extracellular signals, including cytokines, growth factors, stress related proteins etc. [37]. Activation of the pathway causes NF-κB to translocate to the nucleus where it activates several genes involved in cancer formation and progression [23, 37]. Previously, it has been reported that docosahexaonic acid together with a synthetic organoselenium, NO-donating aspirin, and β-lapachone, affects both pathways simultaneously [8, 36, 45]. Also curcumin, the active ingredient of turmeric, can target both the β-catenin and NF-κB pathways [43]. Anti-inflammatory drugs exert their effects through both pathways but little information is available on a simultaneous inhibition in vivo.

In humans nuclear accumulation of β-catenin strongly correlates with tumor size and dysplasia [7, 16] and also its target, cyclin D1, is frequently overexpressed in colon cancers [3]. In this study white currant was able to decrease the accumulation of β-catenin and also presumably the level of cyclin D1, an effect that can also be seen when treating cancer patients with NSAIDs [6]. A reduction in nuclear β-catenin indicates that it can be efficiently degraded, or that activation of the Wnt-pathway has been attenuated, resulting in reduced transcription of targets, such as cyclin D1. Our result is supported by an in vitro study where white currant juice inhibited the growth of cancer cells [4]. In addition to the favorable effects on Wnt-pathway, white currant also reduced nuclear NF-κB protein levels in the adenomas. The targets of NF-κB include several genes related to proliferation and anti-apoptosis [37]. We saw,

however no change in the NF-κB target p53 in the adenomas, but its expression was slightly increased by white currant in the mucosa. Also several drugs are able to inhibit nuclear accumulation of NF-κB, and drug development is underway to target this pathway [25].

During the last few years, there has been an increasing interest in the tumor microenvironment as a factor in cancer formation. It is also widely believed that inflammation plays an important part in developing tumors, and the process of tumor formation has been compared to acute inflammation [19]. The tumor microenvironment is an important target for chemoprevention and potential natural compounds that can target the microenvironment include resveratrol, deguelin, which is a component of the rotenoids, sulphophrane, which in found in brussel sprouts, and apigenin, a flavone [2]. It could therefore be possible that also white currant is able to cause an improvement in the microenvironment. The NF-κB pathway has a major role in the response to pro-inflammatory stimuli [24], and the decrease in NF-κB levels may indicate that white currant was able to reduce inflammation, hypoxia etc in the mucosa surrounding the tumor tissue. Indeed, apoptosis seemed to have been increased in the mucosa of white currant fed mice. Recently, the microenvironment was also suggested to affect the Wnt-pathway and so an improvement of the tumor microenvironment could also explain the reduced nuclear β-catenin seen in this study [14]. However, more studies are needed to test this hypothesis and further analyze the mechanisms of chemoprevention by white currant.

As demonstrated in this study, chemoprevention using natural, non-toxic substances can achieve the same effect as drugs, both in terms of reduction in tumor burden, and inactivation of cell signaling. Attenuation of both β-catenin and NF-κB signaling makes white currant a promising and intriguing agent for colon cancer prevention. The concurrent inhibition of two major pathways in tumourigenesis suggests synergistic and additional effects that could explain the significant chemoprevention seen in this study. As colon cancer is a disease that is closely related to diet it would be of great importance to obtain a deeper understanding on how cells respond to different dietary regimen.

Acknowledgements We wish to thank Marja Carelia for kindly providing the white currants. This study was financially supported by the Ministry of Agriculture and Forestry, the Innovation in Food Programme of the National Technology Agency of Finland, the University of Helsinki, the Finnish Cultural Foundation, the Finnish Graduate School on Applied Bioscience and the Yrjö Jahnsson Foundation.

References

1. Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D (2006) Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J Agric Food Chem 54:980–985
2. Albini A, Sporn MB (2007) The tumour microenvironment as a target for chemoprevention. Nat Rev Cancer 7:139–147
3. Arber N, Hibshoosh H, Moss SF, Sutter T, Zhang Y, Begg M, Wang S, Weinstein IB, Holt PR (1996) Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. Gastroenterology 110:669–764
4. Boivin D, Blanchette M, Barrette S, Moghrabi A, Beliveau R (2007) Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkappaB by edible berry juice. Anticancer Res 27:937–948
5. Boon EM, Keller JJ, Wormhoudt TA, Giardiello FM, Offenhaus GJ, van der Neut R, Pals ST (2004) Sulindac targets nuclear β-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. Br J Cancer 90:224–229
6. Boon EM, Keller JJ, Wormhoudt TA, Giardiello FM, Offerhaus GJ, van der Neut R, Pals ST (2004) Sulindac targets nuclear β-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. Br J Cancer 90:224–229
7. Brabletz T, Herrmann K, Jung A, Faller G, Kirchner T (2000) Expression of nuclear beta-catenin and c-myc is correlated with tumor size but not with proliferative activity of colorectal adenomas. Am J Pathol 156:865–870
8. Choi BT, Cheong J, Choi YH (2003) β-Lapachone-induced apoptosis is associated with activation of caspase-3 and inactivation of NF-κB in human colon cancer HCT-116 cells. Anticancer Drugs 14:845–850
9. Clevers H (2006) Wnt/β-catenin signaling in development and disease. Cell 127:469–480
10. Cooke D, Schwarz M, Boocock D, Winterhalter P, Steward WP, Gescher AJ, Marczylo TH (2006) Effect of cyanidin-3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the ApcMin mouse model of intestinal carcinogenesis—relationship with tissue anthocyanin levels. Int J Cancer 119:2213–2220
11. Duthie SJ (2007) Berry phytochemicals, genomic stability and cancer: Evidence for chemoprotection at several stages in the carcinogenic process. Mol Nutr Food Res 51:665–674
12. van Es JH, Clevers H (2005) Notch and Wnt inhibitors as potential new drugs for intestinal neoplastic disease. Trends Mol Med 11:496–502
13. Ferguson PJ, Kurowska EM, Freeman DJ, Chambers AF, Koropatnick J (2006) In vivo inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. Nutr Cancer 56:86–94
14. Fodde R, Brablez T (2007) Wnt/β-catenin signaling in cancer stemness and malignant behaviour. Curr Opin Cell Biol 19:150–158
15. Gilmore TD, Herscovitch M (2006) Inhibitors of NF-κB signalling: 785 and counting. Oncogene 25:6887–6899

16. Hao X, Tomlinson I, Ilyas M, Palazzo JP, Talbot IC (1997) Reciprocity between membranous and nuclear expression of β-catenin in colorectal tumours. Virchows Arch 431:167–172
17. Harris GK, Gupta A, Nines RG, Kresty LA, Habib SG, Frankel WL, LaPerle K, Gallagher DD, Schwartz SJ, Stoner GD (2001) Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2′-deoxyguanosine levels in the Fischer 344 rat. Nutr Cancer 40:125–133
18. Heinonen M (2007) Antioxidant activity and antimicrobial effect of berry phenolics - a Finnish perspective. Mol Nutr Food Res 51:684–691
19. Huang S, Ingber DE (2007) A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. Breast Dis 26:27–54
20. Hugh TJ, Dillon SA, Taylor BA, Pignatelli M, Poston GJ, Kinsella AR (1999) Cadherin-catenin expression in primary colorectal cancer: a survival analysis. Br J Cancer 80:1046–1051
21. Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J, Törrönen R (1999) Screening of selected favonoids and phenolic acids in 19 berries. Food Res Int 32:345–353
22. Kang SY, Seeram NP, Nair MG, Bourquin LD (2003) Tart cherry anthocyanins inhibit tumor development in Apc(Min) mice and reduce proliferation of human colon cancer cells. Cancer Lett 194:13–19
23. Karin M, Ben-Neriah Y (2000) Phosphorylation meets ubiqtination: the control of NF-κB activity. Annu Rev Immunol 18:621–623
24. Karin M, Greten FR (2005) NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 5:749–759
25. Karin M, Yamamoto Y, Wang QM (2004) The IKK NF-κB system: a treasure trove for drug development. Nat Rev Drug Disc 3:17–26
26. Katsube N, Iwashita K, Tsushida T, Yamaki K, Kobori M (2003) Induction of apoptosis in cancer cells by Bilberry (*Vaccinium myrtillus*) and the anthocyanins. J Agric Food Chem 51:68–75
27. Koponen JM, Happonen AM, Mattila PH, Törrönen AR (2007) Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. J Agric Food Chem 55:1612–1619
28. Kundu JK, Choi KY, Surh YJ (2006) β-Catenin-mediated signaling: a novel molecular target for chemoprevention with anti-inflammatory substances. Biochim Biophys Acta 1765:14–24
29. Lala G, Malik M, Zhao C, He J, Kwon Y, Giusti MM, Magnuson BA (2006) Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. Nutr Cancer 54:84–93
30. Luu HH, Zhang R, Haydon RC, Rayburn E, Kang Q, Si W, Park JK, Wang H, Peng Y, Jiang W, He TC (2004) Wnt/β-catenin signaling pathway as a novel cancer drug target. Curr Cancer Drug Targets 4:653–671
31. Misikangas M, Pajari AM, Päivärinta E, Mutanen M (2005) Promotion of adenoma growth by dietary inulin is associated with increase in cyclin D1 and decrease in adhesion proteins in Min/+ mice mucosa. J Nutr Biochem 16:402–409
32. Misikangas M, Pajari AM, Päivärinta E, Oikarinen SI, Rajakangas J, Marttinen M, Tanayama H, Törrönen R, Mutanen M (2007) Three Nordic berries inhibit intestinal tumorigenesis in Min/+ mice by modulating β-catenin signaling in the tumor and transcription in the mucosa. J Nutr (in press)
33. Moser AR, Pitot HC, Dove WF (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247:322–324
34. Määttä-Riihinen KR, Kamal-Eldin A, Mattila PH, Gonzalez-Paramas AM, Törrönen AR (2004) Distribution and contents of phenolic compounds in eighteen Scandinavian berry spieces. J Agric Food Chem 52:4477–4486
35. Määttä K, Kamal-Eldin A, Törrönen R (2001) Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.). Antioxid Redox Signal 3:981–993
36. Narayanan BA, Narayanan NKD, Desai D, Pittman B, Reddy BS (2004) Effects of a combination of docosahexaenoic acid and 1,4-phenylene bis(methylene) selenocyanate on cyclooxygenase 2, inducible nitric oxide synthase and β-catenin pathways in colon cancer cells. Carcinogenesis 25:2443–2449
37. Pahl HL (1999) Activators and target genes of Rel/ NF-κB trancription factors. Oncogene 18:6853–6866
38. Päivärinta E, Pajari AM, Törrönen R, Mutanen M (2006) Ellagic acid and natural sources of ellagitannins as possible chemopreventive agents against intestinal tumorigenesis in the Min mouse. Nutr Cancer 54:79–83
39. Reeves PG, Nielsen FH, Fahey GB (1993) AIN-93 purified diets for laboratory rodents: final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939–1951
40. Seeram NP, Adams LS, Hardy ML, Heber D (2004) Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. J Agric Food Chem 52:2512–2517
41. Su LK, Kinzler KW, Vogelstein B, Preisinger AC, Moser AR, Luongo C, Gould KA, Dove WF (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science 256:668–670
42. Takayama T, Shiozaki H, Shibamoto S, Oka H, Kimura Y, Tamura S, Inoue M, Monden T, Ito F, Monden M (1996) β-catenin expression in human cancers. Am J Pathol 148:39–46
43. Thangapazham RL, Sharma A, Maheshwari RK (2006) Multiple molecular targets in cancer chemoprevention by curcumin. AAPS J 8:E443–E449
44. Valizadeh A, Karayiannakis AJ, el-Hariry I, Kmiot W, Pignatelli M (1997) Expression of E-cadherin-associated molecules (α-, β-, and γ-catenins and p120) in colorectal polyps. Am J Pathol 150:1977–1984
45. Williams JL, Nath N, Chen J, Hundley TR, Gao J, Kopelovich L, Kashfi K, Rigas B (2003) Growth inhibition of human colon cancer cells by nitric oxide (NO)-donating aspirin is associated with cyclooxygenase-2 induction and beta-catenin/T-cell factor signaling, nuclear factor-kappaB, and NO synthase 2 inhibition: implications for chemoprevention. Cancer Res 63:7613–7618
46. World Cancer Research Fund/American Institute for Cancer Research (2007) Food, nutrition, physical activity, and the prevention of cancer: a global perspective. AIRC, Washington DC
47. Yamamoto Y, Yin MJ, Lin KM, Gaynor RB (1999) Sulindac inhibits activation of the NF-κB pathway. Biol Chem 274:27307–27314
48. Yi W, Fischer J, Krewer G, Akoh CC (2005) Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. J Agric Food Chem 53:7320–7329
49. Yin MJ, Yamamoto Y, Gaynor RB (1998) The anti-inflammatory agents aspirin and salicylate inhibit the activity of IκB kinase-β. Nature 396:77–80
50. Zhao C, Giusti MM, Malik M, Moyer MP, Magnuson BA (2004) Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. J Agric Food Chem 52:6122–6128