Dietary Induction of Colonic Tumors in a Mouse Model of Sporadic Colon Cancer

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Abstract

A defined rodent "new Western diet" (NWD), which recapitulates intake levels of nutrients that are major dietary risk factors for human colon cancer, induced colonic tumors when fed to wild-type C57Bl/6 mice for 1.5 to 2 years from age 6 weeks (two-thirds of their life span). Colonic tumors were prevented by elevating dietary calcium and vitamin D_3 to levels comparable with upper levels consumed by humans, but tumorigenesis was not altered by similarly increasing folate, choline, methionine, or fiber, each of which was also at the lower levels in the NWD that are associated with risk for colon cancer. The NWD significantly altered profiles of gene expression in the flat colonic mucosa that exhibited heterogeneity among the mice, but unsupervised clustering of the data and novel statistical analyses showed reprogramming of colonic epithelial cells in the flat mucosa by the NWD was similar to that initiated by inheritance of a mutant Apc allele. The NWD also caused general down-regulation of genes encoding enzymes involved in lipid metabolism and the tricarboxylic acid cycle in colonic epithelial cells before tumor formation, which was prevented by the supplementation of the NWD with calcium and vitamin D_3 that prevented colon tumor development, demonstrating profound interaction among nutrients. This mouse model of dietary induction of colon cancer recapitulates levels and length of exposure to nutrients linked to relative risk for human sporadic colon cancer, which represents the etiology of >90% of colon cancer in the United States and other Western countries. [Cancer Res 2008;68(19):7803–10]

Introduction

Profound dietary effect on incidence and progression of colon cancer is shown by epidemiologic studies (1, 2), especially rapid shifts in colon tumor incidence as migrant populations adopt new dietary habits (3). Moreover, a defined mouse Western-style diet that qualitatively and quantitatively mimics risk factors in human Western populations (higher fat, lower calcium, and vitamin D_3; 4, 5), accelerates and amplifies intestinal tumor formation in every mouse genetic model of intestinal cancer in which it has been studied, regardless of aggressiveness of disease caused by the genetic mutation (6–9). Data suggest that modifications of this diet, a new Western diet (NWD) encompassing additional nutritional risk factors (lower levels of donors to the single carbon pool and lower fiber), can initiate intestinal tumor formation in the absence of any other genetic or carcinogenic initiator (10).

Here, we assessed tumor formation after feeding the NWD to normal *C57Bl/6* wild-type mice for 1 to 2 years (approximately two-thirds of their life span), the effect of supplementing the diet with individual nutritional components that are at the lower levels in the NWD associated with higher colon cancer risk, and patterns of gene expression in the flat mucosa linked to altered risk, well before tumor development. Colon tumor incidence and frequency tracked with calcium and vitamin D_3 levels in the NWD, as previously reported (10). Furthermore, reprogramming of epithelial cells by the NWD was similar to changes in the flat mucosa of the $Apc1638^{N/+}$ mouse, and the NWD also reduced expression of key genes in the TCA cycle that were reversed by elevating calcium and vitamin D_3.

The link to specific nutrients and to nutrient levels that are risk factors in human colon cancer in this mouse dietary model, coupled with tumor incidence, multiplicity, and length of time to tumor detection, and the potential link to molecular pathways causative for the human disease, suggest this is a model for human sporadic colon cancer, the form of the disease in the overwhelming majority of patients with colon cancer.

Materials and Methods

Mice. Three- to 4-wk-old *C57Bl/6* mice of both sexes from The Jackson Laboratory, housed 10 mice per wire-bottom cage (males and females separately) to prevent coprophagia, were acclimated for 6 wk to AIN-76A diet (11, 12) and a 12 h light/dark cycle, then randomized to dietary groups, 60 mice per group. Mice either continued on AIN-76A, or 6 other diets, *ad libitum*, which varied nutrient intake of calcium and vitamin D_3, choline, methionine, folate, and fiber (Research Diets; Supplementary Table S1). Formulation of these diets has been discussed in detail (4, 10, 13). Mice were sacrificed at 12, 18, and 24 mo for analysis of histopathology, as described (14, 15); 2 females and 2 males from each group were sacrificed at 6 mo for RNA isolation from the large and small intestine, and microarray analysis.

Derivation, genotyping, and intestinal tumor formation in the $Apc1638^{N+/-}$ and $Muc2^{-/-}$ mice, each on a homogeneous Bl/6 genetic background, have been described (16, 17).

RNA isolation and microarray analysis. RNA was prepared from isolated colonic epithelial cells from the flat mucosa of four mice from each genetic/dietary group for determination of gene expression profiles using Affymetrix Mouse 430 2 arrays, as described (18).

Statistical analyses. Comparison of tumor formation on the different diets used the Fisher exact test; analyses were carried out using SAS statistical software (SAS Version 9.1.3; SAS Institute).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Intestinal tumor incidence (% of mice with tumors) and multiplicity (number of tumors/mouse) for animals fed the indicated diets from ages 6 wk to 2 y

Diet	*n*	Intestinal tumors	
		Incidence (%)	Multiplicity
AIN76A	15	27	0.27 ± 0.15
NWD	15	53	0.67 ± 0.19
NWD+Ca/vitD	18	6	0.06 ± 0.06
NWD+folic acid	18	44	0.56 ± 0.17
NWD+choline	18	33	0.44 ± 0.19
NWD+methionine	16	38	0.63 ± 0.27
NWD+fiber	17	35	0.59 ± 0.30

Monte Carlo simulation was used to quantitatively and formally analyze overlaps in gene expression induced by the NWD with those induced by either $Apc^{+/-}$ or $Muc2^{-/-}$. Iterations were carried out as follows: for each condition i (i = 1–3, where 1 = NWD, 2 = Apc, 3 = Muc2), n_i genes were randomly selected without replacement from the pool of 45,037 genes where n_i was the number of genes with a 1.5-fold difference for that condition. The number of genes in the NWD/Apc intersection and the number of genes in the NWD/Muc2 intersection was counted and recorded, the simulation repeated for 10,000 iterations, and an empirical 95% confidence interval constructed for the number of overlapping genes. If the actual number of genes in common fell outside the 95% confidence interval, then the results were in excess of chance, i.e., statistically significant at $\alpha = 0.05$. Based on the simulation results, the empirical 95% confidence interval was calculated to be 77 to 114. The 1,180 overlapping genes between NWD and Apc initiation far exceeded the number expected by chance. Similarly, the empirical 95% confidence interval for NWD/Muc2 intersection was calculated to be 116 to 160, and again, the 906 overlapping genes between NWD and Muc2 far exceeded the number expected by chance.

To determine if the overlap in differentially expressed genes between NWD and $Apc^{+/-}$ differed from the overlap between NWD and $Muc2^{-/-}$, κ coefficients were compared (19), a measure of agreement considered to be corrected for chance agreement. This measures the agreement between two conditions (e.g., NWD and *Apc*) with respect to a 1.5-fold change rated as positive or negative for the 45,037 genes. Two κ coefficients were calculated measuring the agreement between NWD and *Apc* or between NWD and *Muc2*. Because the NWD was common to both κ coefficients, comparison of independent κ coefficients may not be appropriate (19). Therefore, a method similar to that suggested for comparing correlated κs using the bootstrap technique was used (20). Bootstrap samples were drawn with replacement from each of the three sets of genes (NWD, *Apc*, and *Muc2*). Each set consisted of an indicator of zero or one for each of the 45,037 genes, where one indicated that the gene showed a 1.5-fold difference when compared with the control. The κ coefficient was calculated between the bootstrapped NWD and Apc samples (κ1) and between the bootstrapped NWD and Muc2 samples (κ2). The difference between the κ statistics was calculated. Based on 10,000 iterations, the agreement between NWD and Apc as measured by the κ coefficient was 0.53 and the agreement between NWD and Muc2 was 0.29. Therefore, the difference between κ coefficients was 0.24. Based on the bootstrapped results, the empirical 95% confidence interval for the difference in κ coefficients was 0.22 to 0.25. Because the confidence interval did not include zero, we concluded that the agreement between NWD and Apc is greater than the agreement between NWD and Muc2.

Results and Discussion

The NWD increases lipid content, and decreases calcium and vitamin D_3, fiber, and methyl-donor nutrients (folic acid, choline, and methionine) to nutrient-density levels associated with risk for colon cancer that are consumed by large segments of human Western populations. At 24 months, overall intestinal tumor incidence approximately doubled in *C57Bl/6* mice fed the NWD compared with those fed AIN-76A and intestinal tumor multiplicity increased 1.5 fold (P = 0.079; Table 1). A clearer relationship between tumor development and diet was seen for the large intestine. Although spontaneous colonic tumors were rare in *C57Bl6* mice fed AIN-76A for up to 2 years, colonic tumor incidence and multiplicity substantially increased in mice fed the NWD, with 27% of mice exhibiting one to several tumors after two-thirds of their life span (i.e., 2 years; Fig. 1*A* and *B*). Tumors per tumor-bearing mouse were 1.0, 1.25, and 0 for mice fed AIN-76A, NWD, and NWD+calcium/vitamin D_3, respectively, for 2 years. Of the total of 10 colonic tumors found in mice fed the NWD for 18 or 24 months, 3 were microadenomas, 5 flat adenomas, 1 tubular adenoma, and there was 1 invasive carcinoma.

The effect of raising each nutrient at reduced level in the NWD to the nutrient density equivalent of the upper intake range for human western populations was tested (Supplementary Table S1). Neither methionine, choline, folate, nor fiber raised individually

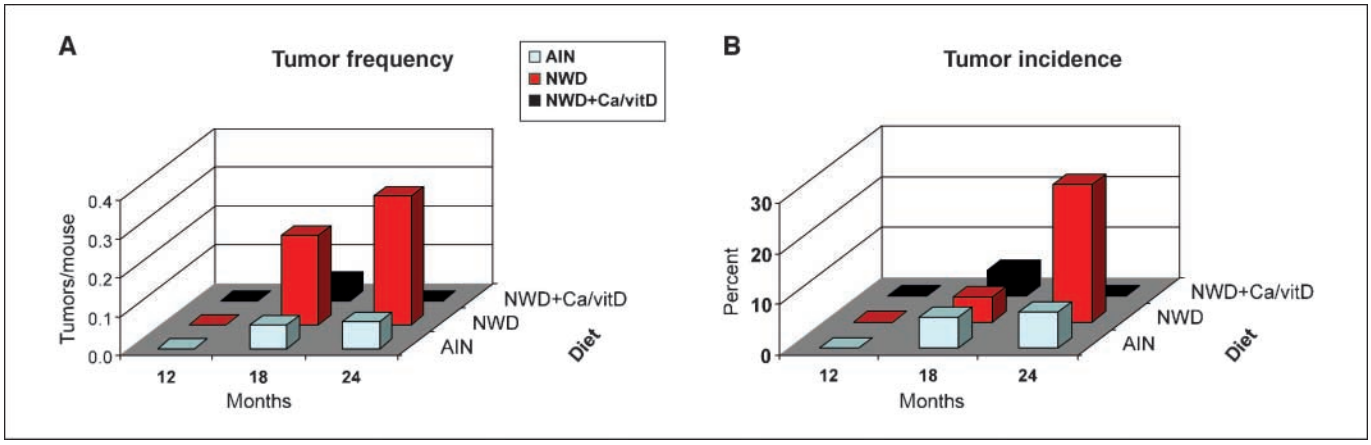

Figure 1. Number of colonic tumors per mouse (*A*) and percent of mice with colonic tumors (*B*) in groups of *C57Bl/6* mice (n = *12–22*) fed a control-defined diet (*AIN-76A*), the NWD, and the NWD supplemented with additional calcium and vitamin D_3 (*NWD+Ca/vitD*) after weaning until sacrifice at 12, 18, or 24 mo.

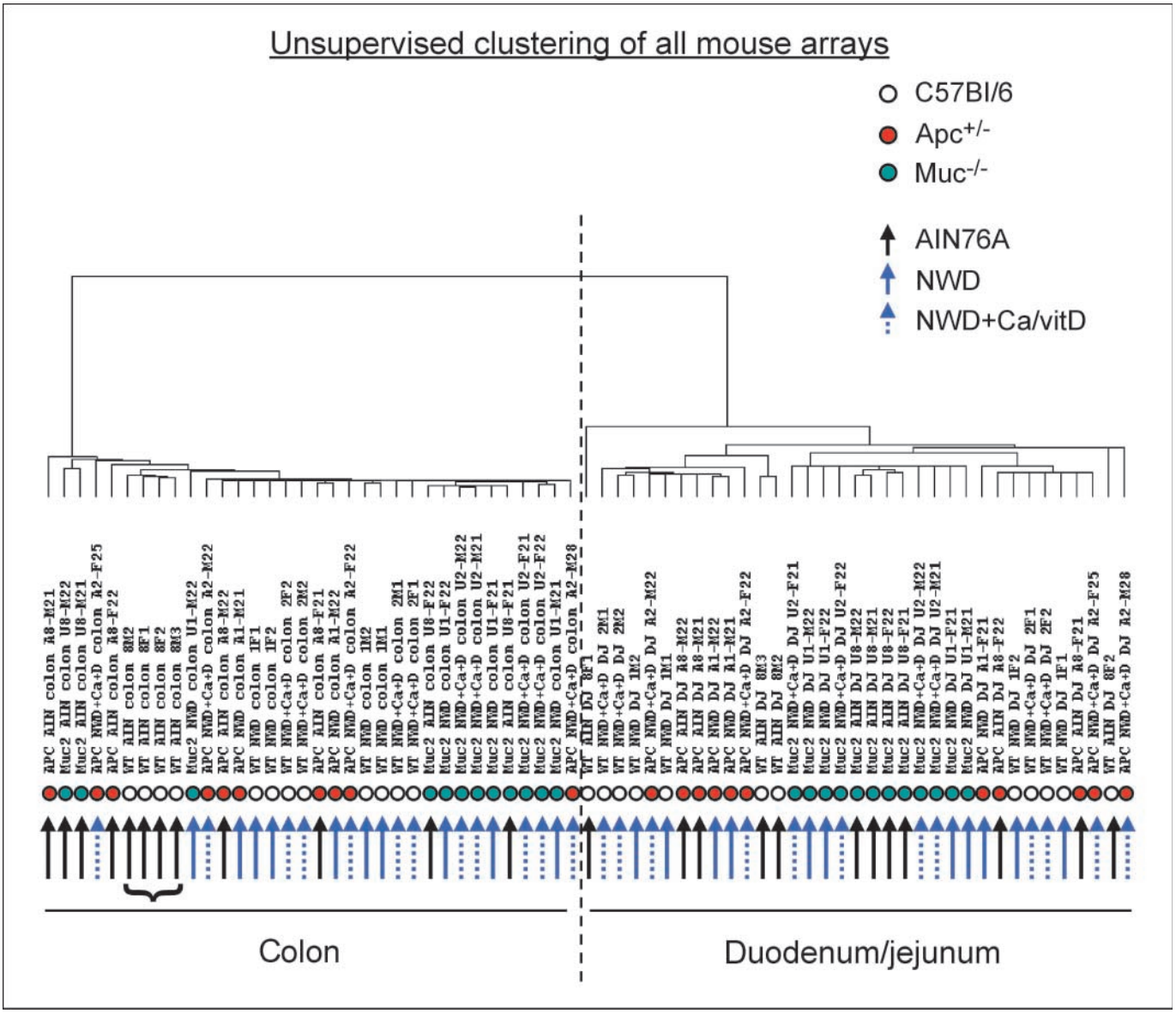

Figure 2. Unsupervised clustering of each mouse in a matrix of 9 genetic/dietary groups (*C57Bl/6, Apc1638$^{N/+}$*, or *Muc2$^{-/-}$* mice fed either AIN76A, NWD, or NWD+calcium/vitamin D) based on the entire gene expression database for the duodenum/jejunum and the colon.

altered colon tumor incidence induced by the NWD over 2 years (data not shown). However, elevating calcium and vitamin D_3 from the equivalent of 220 mg and 50 IU in a 2,000 kcal/day human diet, respectively, to the human equivalent of 3,000 mg and 1,000 IU per day, prevented colonic tumor formation in the mouse by the NWD (Fig. 1*A* and *B*; $P < 0.01$; tumor multiplicity, 24 months; NWD versus NWD+calcium/vitamin D_3).

Using exact methods, colonic tumor incidence differed significantly with respect to diet at 24 mo ($P = 0.04$). Risk of tumor formation for mice fed the NWD was much greater than risk on the control AIN76A diet (odds ratio, 4.8) or for animals maintained on the NWD supplemented with additional calcium and vitamin D_3 (odds ratio, 7.7). However, the lower risk diets—AIN76A and NWD+calcium/vitamin D_3—were approximately equivalent (odds ratio of 0.8 for comparison of tumor numbers). Thus, colonic tumor formation stimulated by the NWD is prevented by supplementing the diet with calcium and vitamin D_3 to levels equivalent to those chemoprotective for human colon cancer (21–26), despite the other remaining nutrient risk factors (i.e., higher fat, lower methyl donors, and fiber).

Investigation of mechanisms underlying increased probability of tumors caused by the NWD was challenging for theoretical and practical reasons. Only 1 to several tumors developed in 27% of the mice over 2 years, a time during which the mucosa seemed grossly and functionally normal. Thus, of the $\sim 10^{12}$ cell divisions in the colonic mucosa over this period, only an exceedingly small percentage gave rise to cells that initiated tumor formation. This is a strength of this model because it recapitulated the human population, where screening endoscopy of individuals who have consumed diets with similar risk factors for approximately two-thirds of their life span (5–6 decades) uncovers only a single colonic tumor in 15% to 20% of individuals (27). However, this

suggests that changes establishing risk for tumor formation in this model and in human sporadic cancer may be rare, subtle, and drive tumor formation in a stochastic manner.

Therefore, we adopted a strategy of unbiased gene expression profiling analyzing patterns of expression of large numbers of sequences, and pathway analysis, rather than focusing on individual molecules. Experimentally, this approach can reveal perturbations linked to diet that may subtly modulate probability of tumor initiation and/or progression. Theoretically, this may more accurately reflect mechanisms that establish relative risk in the population than focusing on a single gene or molecule.

Reprogramming of epithelial cells from the flat colonic mucosa induced by diet was analyzed after 6 months of feeding. Cells turn over in the mucosa every 3 to 4 days. Therefore, this period of dietary exposure is sufficient for colonic epithelial cells to reflect alterations due to the different diets fed from weaning but is well before tumor detection at 18 to 24 months (Fig. 1*A* and *B*). For comparison, we also investigated expression profiles for the colon and small intestine of *Apc1638*$^{N/+}$ and *Muc2*$^{-/-}$ mice. Each of these models also develops a low number of colonic tumors, but through fundamentally distinct mechanisms: reduction of the inherited *Apc* mutation to homozygosity in *Apc1638*$^{N/+}$ mice increases β-catenin-Tcf signaling, whereas targeted inactivation of *Muc2* does not (14, 17, 28, 29). Four mice were analyzed from each of the nine genetic/dietary groups (*C57Bl/6, Apc1638*$^{N/+}$, or *Muc2*$^{-/-}$ fed AIN-76A, NWD, or NWD+Ca/vit.D$_3$) for both small

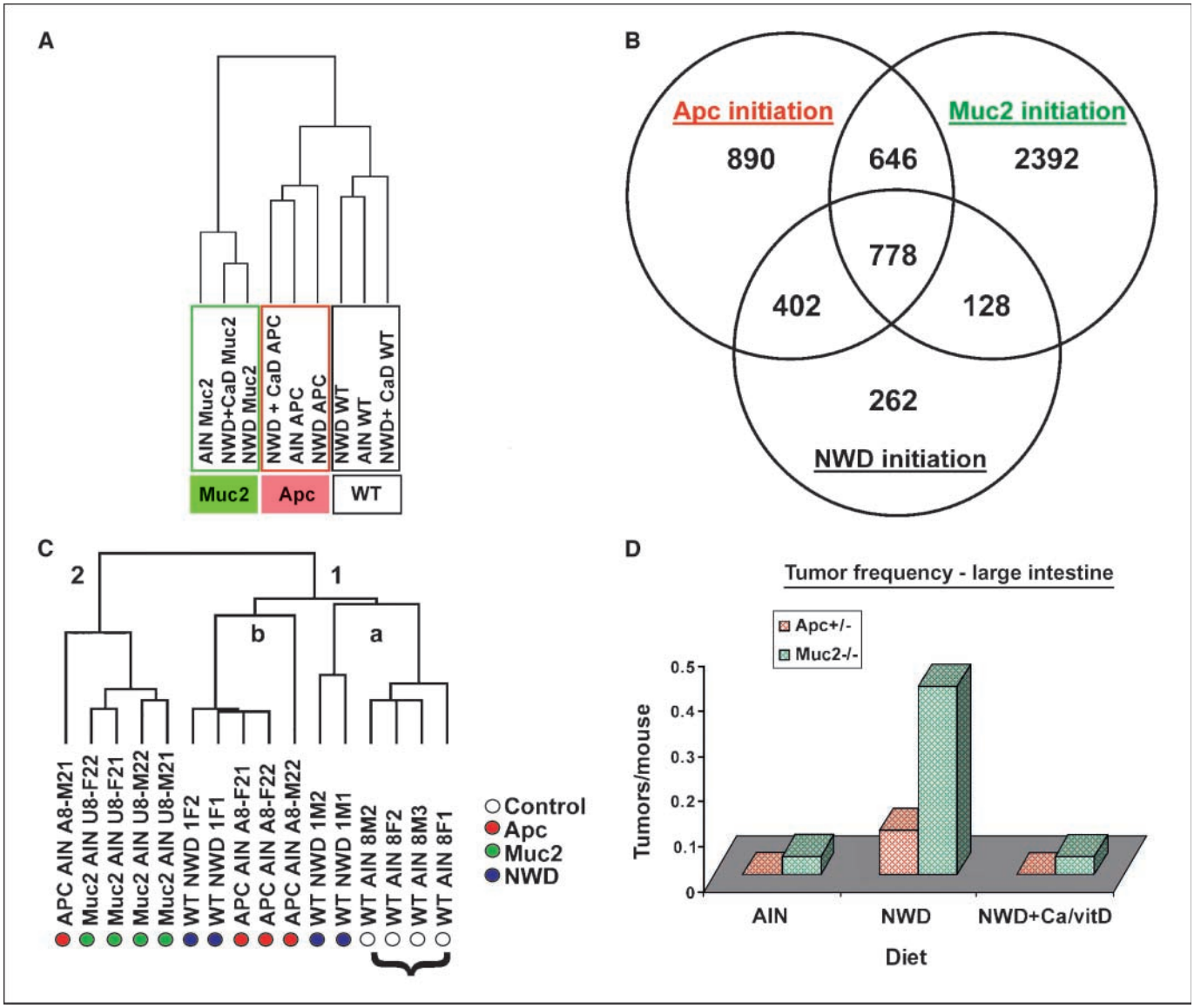

Figure 3. *A* to *C,* analysis of gene expression in the colonic mucosa of *C57Bl/6, Apc1638*$^{N+/-}$, and *Muc2*$^{-/-}$ mice fed either AIN-76A, NWD, or NWD supplemented with calcium and vitamin D$_3$ from ages 6 wk and sacrificed at 6 mo. *A,* the mean for each probe set (i.e., "gene") was calculated for the four mice in each genetic-dietary group, and unsupervised clustering of the means was done as described (43). The number of probe sets for which the mean expression level was increased or decreased in expression by 50% (1.5-fold) was then determined for three comparisons: *C57Bl/6* mice fed the NWD compared with the same genotype fed AIN-76A control diet (dietary tumor induction); the *Apc1638*$^{N+/}$, or the *Muc2*$^{-/-}$ mice, fed AIN76A diet, in each case compared with the *C57Bl/6* mice fed the AIN-76A diet (two mechanisms of genetic initiation). Overlaps among these comparisons are illustrated in the Venn diagram in *B* with the inserted number of probe sets differentially expressed for the various overlaps. *C,* unsupervised clustering using the data for each of the mice for the 5,498 probe sets that differ in at least one of the three comparisons made (dietary, *Apc*, or *Muc2* initiation). *D,* the tumor frequency in the large intestine for *Apc1638*$^{N+/-}$ or *Muc2*$^{-/-}$ mice fed AIN76A, NWD, or the NWD supplemented with calcium and vitamin D for 6 mo. *WT,* wild-type.

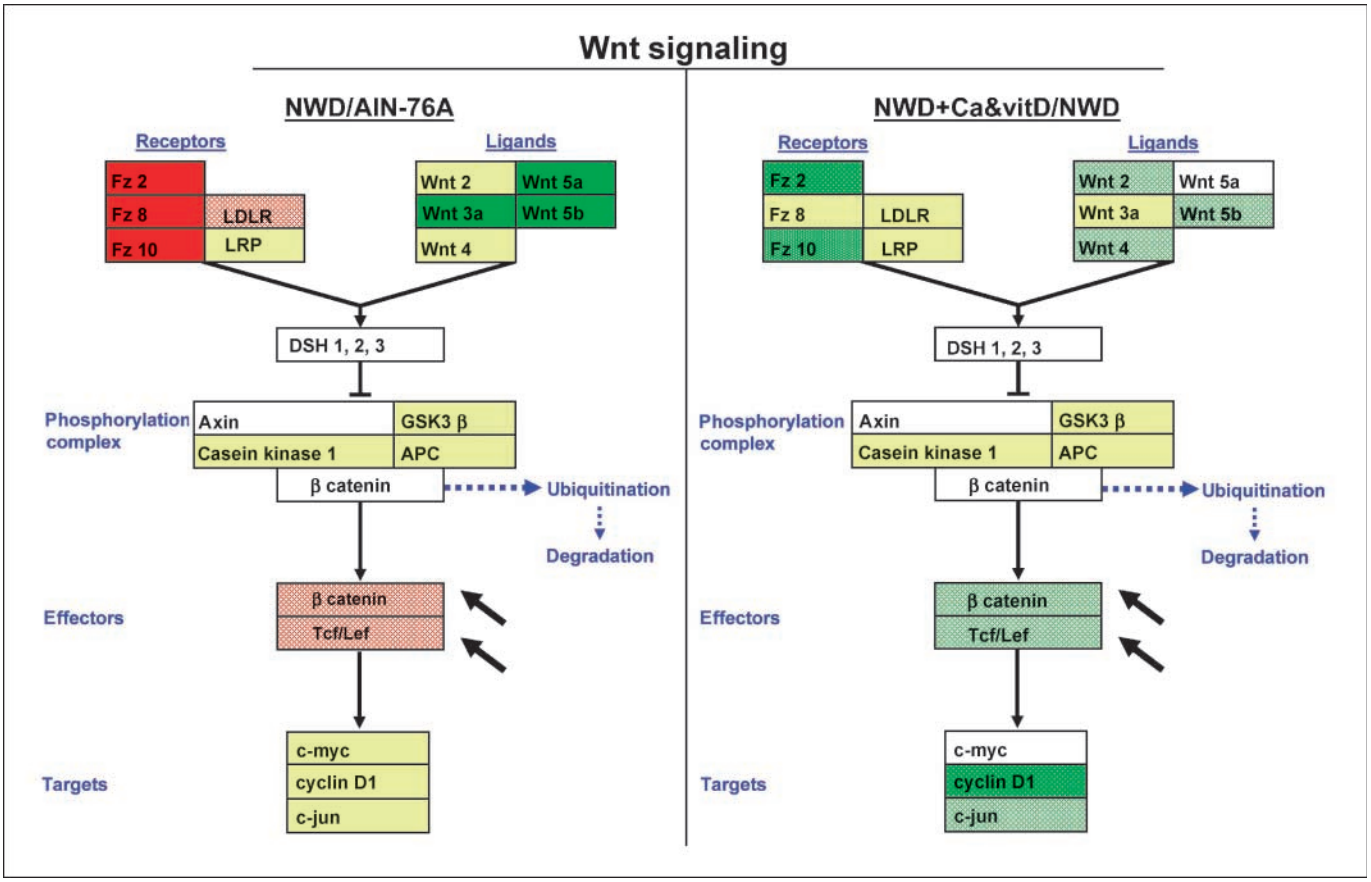

Figure 4. Changes in expression of genes in the Wnt signaling pathway as determined by Affymetrix gene expression profiling for *C57Bl/6* mice fed the NWD compared with mice fed AIN-76A (*A*), or the NWD supplemented with calcium and vitamin D_3 compared with mice fed the NWD (*B*). Data were mapped onto the Wnt pathway using GenMAPP (44), using a pathway map originally generated by Dahlquist and Breymer at GenMapp.org.[7] A simplified map was then drawn to focus on those sequences that were altered in expression in the pathway. *Green and red coloring,* sequences that are decreased or increased in the comparisons, respectively, with the intensity of color proportional to the extent of change.

and large intestine. Regardless of diet or genotype, the gene expression data clustered the mice in two separate organ site branches, reflecting the distinct histology and function of these intestinal tissues (Fig. 2).

We focused on the colon data because diet was more clearly linked to large intestinal tumor formation. Clustering of the mean expression data for each of the 45,037 noncontrol probe sets (i.e., "genes") on the arrays showed that the primary separation of the groups reflects genotype, and only within each genotype are dietary effects noted (Fig. 3*A*), despite the fact that diet has a major effect on colonic tumor formation (Fig. 1 and below). The separation of all of the *Apc1638*$^{N/+}$ dietary groups from all of the *C57Bl/6* dietary groups suggests that inheritance of a single mutant *Apc1638* allele generates alterations in the mucosa even before focal loss of the wild-type *Apc* allele and tumor development. Moreover, clustering indicated that the *C57Bl/6* flat colonic mucosa is more similar to the *Apc1638*$^{N/+}$ than to the *Muc2*$^{-/-}$ mucosa, a reflection of both the subtle effects of inheritance of a single mutant *Apc* allele (30, 31), and the histologic abnormalities present in the mucosa of the *Muc2*$^{-/-}$ mice (17).

We next compared nutritional initiation (*C57Bl/6* mice fed NWD compared with *C57Bl/6* mice fed AIN-76A control diet), to the two distinct mechanisms of genetic initiation (the *Apc1638*$^{N/+}$ mice compared with the *C57Bl6* mice, each fed control diet; and the *Muc2*$^{-/-}$ mice compared with the *C57Bl/6* mice, each fed control diet).

Seventy-five percent of NWD-induced gene expression changes in the colon overlapped with similar changes induced in the mucosa by the *Apc* mutation (Fig. 3*B*), but only 57% overlapped with changes induced in the mucosa by the homozygous *Muc2*$^{-/-}$ mutation (Fig. 3*B*). Of 66 genes altered in opposite directions in the 2 models of genetic initiation, 35 showed no change in nutritional initiation, but 26 were altered in expression by NWD as they were by *Apc* mutation, whereas only 5 were altered as they were by *Muc2* mutation (data not shown).

We term the 5,498 genes that change in expression in at least one of these comparisons (Fig. 3*B*), the "initiator gene subset." On the basis of this gene subset, mice clustered into two main branches (Fig. 3*C, 1* and *2*), with branch 1 subdividing into 1-a and 1-b. All of the wild-type *C57Bl/6* control mice clustered together on branch 1-a (*bracket*). Two of the four *C57Bl/6* mice fed the NWD also clustered on 1-a, with the other two clustered with three of four of the *Apc1638*$^{N/+}$ mice on 1-b. This division of the wild-type mice on NWD between sub-branches 1-a and 1-b is likely because the NWD is the same as AIN-76A in terms of many important nutrients that influence gene expression patterns, and because only 27% of the mice developed colon tumors when fed the

[7] http://www.genmapp.org

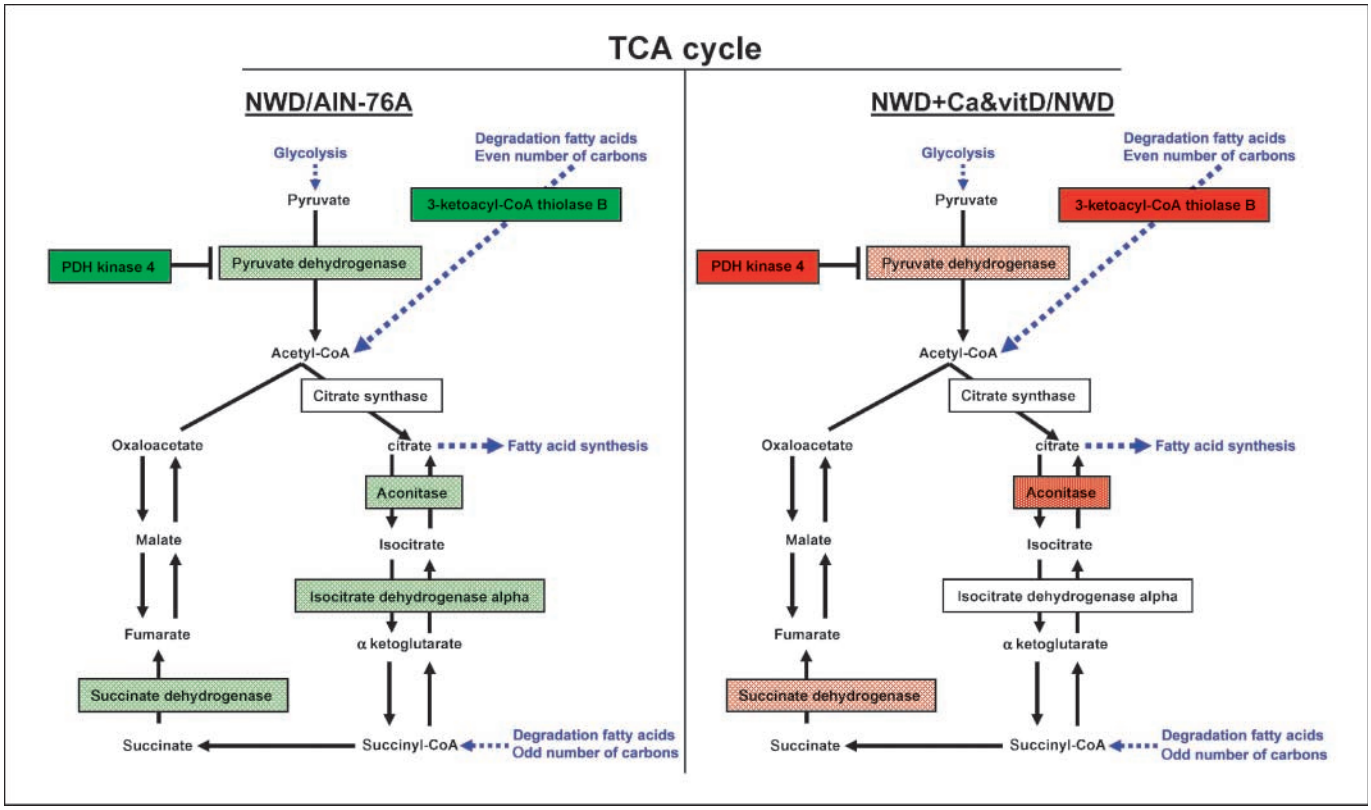

Figure 5. Changes in expression of genes that encode enzymes of the tricarboxylic acid cycle as determined by Affymetrix gene expression profiling for *C57Bl/6* mice fed the NWD compared with mice fed AIN76A (*A*), or the NWD supplemented with calcium and vitamin D_3 compared with mice fed the NWD (*B*). Data were mapped onto the TCA cycle using GenMAPP (44), from a pathway map originally generated by Dahlquist and Breymer at GenMapp.org; *green and red coloring,* sequences that are decreased or increased in the comparisons, respectively, with the intensity of color proportional to the extent of change, as shown in the legends in the figure.

NWD. Therefore, alterations in gene expression in the colonic mucosa may be variable and stochastically linked to tumor formation (*see below*). Of note, all four of the *Muc2* mice fed the control diet clustered on the separate major branch #2, emphasizing that loss of Muc2 expression reprograms the epithelial cells of the colon in a way that differed markedly from either the NWD or inheritance of an Apc mutation.

Monte Carlo simulation showed significant overlap between the gene sets altered in expression in the wild-type mice by the NWD and sets altered in expression in either $Apc^{+/-}$ or $Muc2^{-/-}$ mice ($P < 0.05$; see Materials and Methods). Comparison of κ coefficients coupled with a bootstrap technique (Materials and Methods) showed that the agreement between the NWD and the $Apc^{+/-}$-induced changes were greater than the agreement between the NWD- and the $Muc2^{-/-}$-induced changes. Thus, rigorous statistical analyses support the conclusion, based on clustering of the mice (Fig. 3), that the NWD-induced reprogramming of colonic cells is much more similar to the reprogramming induced by inheritance of an *Apc* mutation than that induced by the *Muc2* mutation.

Analysis of similar gene expression data bases for the small intestine again showed that the NWD induced changes in gene expression profile more similar to the changes in $Apc^{+/-}$ mice compared with $Apc^{+/+}$ than to $Muc2^{-/-}$ compared with $Muc2^{+/+}$ (data not shown).

The NWD increased colon tumors 5-fold when fed to the $Muc2^{-/-}$ mice but <2-fold in $Apc1638^{N/+}$ mice (Fig. 3*D*). This is consistent with the expression data that show the NWD diet complements the changes in the $Muc2^{-/-}$ mice but is more limited to replicating many of the effects of the *Apc* mutation. In each case, calcium/vitamin D_3 supplementation prevented the NWD-stimulated increase.

In tumors in $Apc1638^{N/+}$ mice, loss of the wild-type allele activates Wnt signaling (14). However, it is not clear if there is haplo-insufficiency of *Apc* activated Wnt signaling in the flat mucosa of $Apc1638^{N/+}$ mice. Furthermore, although the NWD and the inheritance of a mutant *Apc* allele in the mucosa alter gene expression profiles similarly, this does not mean that Wnt signaling is altered by the NWD. Figure 4*A* shows that a number of genes in the Wnt signaling pathway were altered in expression by the NWD, although only to a modest extent and not statistically significantly for any one sequence. Because colonic tumor formation inversely followed calcium/vitamin D_3 levels in the NWD (Fig. 1), this was compared with changes in this pathway in mice fed the NWD + calcium/vitamin D_3 relative to mice fed the NWD (Fig. 4*B*). Particularly interesting were sequences for which expression tracked with calcium/vitamin D_3 levels and, hence, probability of tumor formation. This included the Wnt ligands Fz 2 and 10, and β-catenin and Tcf (Fig. 4, *arrows*), the latter encoding the two components of the major transcriptional effector of Wnt signaling. Each was only modestly elevated and reversed by the NWD and by addition of calcium/vitamin D_3, respectively, but the coincidence of these increases and reversals are striking.

This approach was extended to 680 UniGene clusters that either increased or decreased significantly in expression in the colon by NWD but which significantly shifted back when NWD was supplemented with calcium and vitamin D_3. In this 680 gene

subset, 20 of 112 (18%) predefined functional groups were enriched for changes in expression that tracked with calcium/vitamin D_3 levels and tumor formation (Supplementary Table S2). Consistent with adaptation of the mucosa to altered fat level in the NWD, six of these enriched functional groups (Supplementary Table S2) reflected energy and lipid metabolism (i.e., electron transport, fatty acid metabolism, glycolysis, lipid biology, oxidative phosphorylation, and tricarboxylic acid cycle). Of the 58 genes in these 6 groups that were altered significantly in expression, most genes (i.e., 46 of 58, or 79%) decreased in expression when mice were fed the NWD, and showed a complementary increase when the NWD was supplemented with calcium/vitamin D_3; in contrast, 21% increased on the NWD and decreased with supplemental calcium/vitamin D_3. To complement this, we analyzed the entire data base by parametric analysis of gene set enrichment ("PAGE"; ref. 32), using fold change as the metric between groups performed for GO Biological terms, Molecular function terms and Pathways, using "Gazer" to curate the sets of genes examined and to calculate enrichment scores (33). This also identified the TCA cycle as having a significant negative Z score in comparing the NWD to the AIN76A diet in C57Bl/6 mice ($P < 0.002$) and a significant positive Z score in comparing the NWD+calcium/vitamin D diet to NWD ($P < 0.001$). Thus, there may be profound interaction of nutrients in determining profiles of intermediary metabolism, illustrated in Fig. 5*A*. Genes in the TCA cycle that were down-regulated by NWD, included pyruvate dehydrogenase ($P = 0.0002$), which encodes the enzyme that generates acetyl-CoA from carbohydrate metabolism, succinate dehydrogenase ($P = 0.0019$), an alternate point for entry of electrons into the TCA cycle, and aconitase ($P = 0.0007$), which catalyzes conversion of citrate to isocitrate, which when decreased can supply greater cytoplasmic levels of citrate for lipid synthesis, an important feature of tumor formation. Moreover, each of these is elevated by supplementation of NWD with calcium and vitamin D_3 (Fig. 5*B*). Furthermore, the gene that encodes 3-ketoacyl-CoA thiolase B, which catalyzes generation of acetyl CoA from lipids, is also down-regulated by NWD and elevated back toward control levels by calcium and vitamin D_3 supplementation (Fig. 5*A* and *B*). Thus, generation of acetyl CoA for the TCA cycle from both carbohydrates and lipids may be compromised in mice fed NWD, suggesting that glycolytic metabolism may be elevated in the colonic mucosa in which dietary factors have elevated the probability of tumor formation. Although increased glycolytic metabolism was recognized as a characteristic of tumors over 50 years ago (34), and is a characteristic of human colon tumors, it is intriguing that such metabolic alterations may precede, and be a risk factor for, tumor formation. Possible mechanisms by which such metabolic shifts may drive tumorigenesis have been discussed (35–37).

In investigating mechanisms of increased probability of tumor formation, it is important to be clear regarding expectations. Only 1 in 4 mice develops 1 to several colonic tumors over a period of 1.5 to 2 years, during which time the mucosa seems to be normal, as is the case in the general human population. This differs from genetic models, in which the introduction of a genetic alteration usually alters the vast majority of the cells in the target tissue in a predefined way. Even in such genetic models, tumor penetrance is often not high, indicating the stochastic nature of tumor development. In this dietary model, we believe the stochastic nature of tumor initiation, promotion, and progression is more profound and is dependent on multiple events that only rarely occur coincidently or in the correct sequence. For example, in analysis of gene expression profiles, the wild-type mice fed the control diet always cluster together, whereas other genetic and/or dietary groups cluster less consistently. Thus, we hypothesize that genetic and dietary factors alter gene expression in a way that generates significant variation, and that increased probability for tumor formation may reside in the integration of many modest genetic and/or dietary perturbations of pathways that establish and maintain normal homeostasis, rather than in a single dominant alteration. Similarly, Bodmer and colleagues (38) have described how multiple independent genetic factors, none of which are significant individually in the human population, can combine to determine higher risk for tumor development.

Despite this inherent complexity, we showed that the NWD reprogrammed epithelial cells of the mucosa in a way similar to that generated by inheritance of an *Apc* mutation. This involved altered expression of genes in the Wnt signaling pathway, but other important functions of *Apc*, including regulation of genomic stability, could contribute to the disruption of homeostasis in this exquisitely complex and balanced tissue (39). These data also indicated that, in at least some respects, Apc may exhibit haploinsufficiency of a single wild-type allele. Regarding this, a decrease in APC protein of ~85% is necessary for formation of one polyp per mouse (31). Although loss of a single *Apc* allele might, on average, reduce Apc expression by less than this (i.e., ~50%) normal fluctuations in expression of a gene already reduced by an average of 50% can frequently bring transient, or stochastic, changes to much lower levels (40). Depending on the timing and coincidence of such decreases in relation to the expression of other genes in a cell, this might be sufficient to trigger initial events that can cascade into disruption of homeostasis and tumor formation.

In summary, we dissected a dietary model of mouse colon cancer that in many ways recapitulates the etiology and pattern of formation of human sporadic colon cancer, the form of the disease responsible for >90% of cases of colon cancer in the United States and other Western countries. The only other model of dietary induction of solid tumor formation in the rodent is hepatocellular carcinoma induced by methyl and choline deficiency in the rat, but this is not effective in the mouse (41, 42). In the model analyzed here, relative levels of calcium and vitamin D_3 are major determinants of risk for colon cancer. For reference and analysis, the entire gene expression database for all the dietary/genetic groups can be accessed as Supplementary Data and at our Web site.[8] Further investigations will determine how these gene expression changes come about, the distribution of these events as a function of time of dietary exposure and in relation to the architecture and functional compartments of the colonic crypt, and how they elevate probability of tumor formation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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