

Different Gene Expression of Skin Tissues Between Mice with Weight Controlled by Either Calorie Restriction or Physical Exercise

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Cancer prevention by weight control via dietary calorie restriction (DCR) and/or exercise has been demonstrated in animal models. To understand the underlying mechanisms, we compared phorbol ester (TPA)-induced gene expression profiles in DCR- or exercise-treated mouse skin tissues. SENCAR mice were randomly assigned to one of the following groups: *ad libitum*-fed sedentary control, *ad libitum*-fed exercise (AE), exercise but pair-fed at the amount of the control (PE), and 20% DCR. After 10 weeks, both body weight and fat composition significantly decreased in the DCR and PE groups compared with the controls. Weight loss was not observed in the AE group due, at least in part, to increased food intake. Among 39,000 transcripts with 45,101 probe sets measured by Affymetrix microarray, we identified 411, 110, and 67 genes that showed ≥ 1.5 -fold and significant changes by DCR, AE, and PE, respectively. Gene ontology showed a profound impact on gene expression by DCR in 21 biologic process categories. Although PE and AE had a moderate impact on gene expression, the similarity of gene expression pattern altered by PE was relatively closer to DCR, whereas AE was closer to the control. The results of 22 cancer-related gene expression patterns, especially for certain oncogenes, further supported that PE appeared to be a better alternative than AE to DCR-like cancer prevention. The impact on gene expression pattern was associated with the effect on weight loss (i.e., DCR \gg PE $>$ AE). Overall, this study demonstrated for the first time that

weight control via decreasing energy intake or increasing energy expenditure resulted in the different modes of gene expression. DCR showed profound inhibitory impact on the expression of genes relevant to cancer risks. Furthermore, exercise along with limited calorie intake appears to be a better method for reducing weight and cancer risk compared with exercise alone. *Exp Biol Med* 232:473–480, 2007

Key words: weight control; gene expression; calorie restriction; exercise; mice

Introduction

The National Health and Nutrition Examination Survey in 1999–2000 indicates growing obesity rates in American adults over the past 20 years (1). Case-control and prospective cohort studies have identified obesity as a risk factor for many types of cancer, including colon, breast, endometrial, kidney, and esophageal cancers (2). High prevalence of overweight and obesity can be explained by a lifestyle characterized with overconsumption of calorie combined with low physical activity (3). There is ample evidence that weight control via decreasing calorie intake and increasing physical activity reduces cancer risk. The International Agency for Research in Cancer of the World Health Organization convened an International Working Group in 2001 and concluded that limiting weight gain during adult life reduces the risk of a number of different types of cancer and that increasing physical activity prevents cancer (4).

Studies conducted in animal models indeed demonstrated cancer prevention by weight control via dietary calorie restriction (DCR) or exercise. DCR conducted in lean rodents was found to prevent many types of cancers, including mammary, liver, colon, skin, pancreas, and leukemia (5–10). Exercise with or without DCR also was reported to have a potential protection against tumor incidence (11–14). However, it was noted that exercise

This study is supported by an Innovative Research Grant from the Terry Johnson Center for Basic Cancer Research, Kansas State University, grant COBRE P20 RR15563, and by grant R01 CA106397 from the National Institutes of Health.

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Received March 3, 2006.
Accepted October 31, 2006.

1535-3702/07/2324-0473\$15.00
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alone might not consistently result in cancer prevention (15–17).

Although the mechanisms of DCR and/or exercise for cancer prevention are not clear, some explanations such as alterations of hormone metabolism, hormone-related cellular signaling, oxidation status, DNA repair, apoptosis, and oncogene expression, have been postulated (18–20). Microarray analysis has been applied to cancer prevention experiments by DCR to explore the plausible mechanisms (21–28). However, relatively few data have been published regarding the comparison of gene expression between DCR and exercise, and to date there is no information concerning the gene expression profiles in the combination of dietary calorie intake and energy expenditure for cancer prevention.

To determine the mechanisms underlying such complex relationships between body weight, calorie intake, physical activity, and cancer risk, we applied a genomic microarray analysis to the skin tissues of mice that were controlled in body weight by DCR, exercise, and exercise combined with limited calorie intake. The gene expression profiling was compared, and certain phorbol ester (TPA)-induced cancer-related genes were further exploited.

Materials and Methods

Animal and Animal Treatment. Fifty-two female SENCAR mice at 8 weeks of age with body weights averaging 30 ± 2 g were purchased from the National Institutes of Health (Frederick, MD). Mice were randomly assigned to one of the following four groups: *ad libitum*-fed sedentary control, *ad libitum*-fed exercise (AE), exercise but pair-feeding at the same amount as *ad libitum*-fed sedentary counterpart (PE), and 20% DCR. The 20% DCR diet was formulated by the Harlan Teklad (Madison, WI), containing 20% less total calorie from carbohydrates and fat in comparison with the basal AIN-93 diet, and having extra protein and essential micronutrients to maintain a same level as the basal diet. The amount of the food that each control mouse consumed was recorded weekly and averaged to determine the amount of the following week for DCR and pair-feeding consumption. A zero-grade, motor-driven, adjustable-speed rodent treadmill (Boston Gears, Boston, MA) was used to exercise the mice at 0.5 mph for 60 mins per day, 5 days per week, for 10 weeks. To take into account the biologic clocks of nocturnal mice, we adjusted the light cycle to run nighttime exercise at 0400–0500 hrs. The mice were put on a progressive training program starting at 10% of target exercise duration time and progressing to 100% of the target time by the end of the second week. Mice were housed individually in an environmentally controlled room maintained at $24^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ and 80% relative humidity with a 12:12-hr light:dark cycle starting at 1200 hrs. Body weights were recorded weekly. Body composition was monitored in the last week by a dual-energy x-ray absorptiometer scan using small-animal software (v5.6, Prodigy; Lunar-General Electric, Milwaukee, WI). At the

end of the experiment, when mice were about 20 weeks of age, the dorsal skin of the mice was shaved and topically treated once with TPA at 3.2 nmol. Mice were sacrificed by decapitation 2 hrs after TPA treatment. The 2-hr period of TPA treatment was selected based on a time course study reported previously by Przybyszewski *et al.* (29). The dorsal skin samples were snap frozen in liquid nitrogen and kept at -80°C until further analysis.

Microarray Analysis. Microarray analysis was processed by the Microarray Core of the Mental Retardation Research Center at the University of Kansas Medical Center (Kansas City, KS). Four mice from each treatment group were used to obtain mouse skin tissues. Each mouse skin tissue (0.4 μg) was homogenized in 1 ml TRIZOL reagent (Invitrogen, Carlsbad, CA) and the total RNA was extracted and precipitated by chloroform and isopropanol. Pellet RNA was dissolved in diethyl pyrocarbonate water and further purified by using the RNeasy cleanup kit (Qiagen, Valencia, CA). The quantity and quality of RNA were measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA (10 μg) then was annealed with 100 pmol T7(dT)24 at 70°C for 10 mins. The annealed mRNA was reverse transcribed into cDNA using the Superscript Choice System kit (Invitrogen Corp.). Biotinylated antisense cRNA was prepared using the Enzo BioArray High-Yield RNA Labeling kit (Enzo Diagnostics, Farmingdale, NY). After purification of labeled cRNA using the RNeasy RNA Purification Mini kit (Qiagen), 20 μg biotin-labeled cRNA was incubated in fragmentation buffer (40 mM Tris-acetate, 100 mM potassium acetate, 30 mM magnesium acetate, pH 8.1) at 94°C for 35 mins. The labeled cRNA then was applied to a GeneChip Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA) containing 39,000 transcripts with 45,101 probe sets. The GeneChip was hybridized, washed, and scanned using Affymetrix equipment and protocols.

Microarray Data Analysis. Microarray images were quantified by using GeneChip operating software 1.0 (GCOS 1.0; Affymetrix). The detection calls of the probe sets were determined using default settings (α_1 , 0.04; α_2 , 0.06; δ , 0.015; scale factor, 1.0; norm factor, 1.0). GeneSpring software (Agilent Technologies) and Access Database (Microsoft Windows; Microsoft, Seattle, WA) were used to do data analysis. Scatter plot and condition trees were analyzed by GeneSpring Software, and distance of gene expression profile was obtained from GeneSpring and compared among groups. Data were first scaled to the same average intensity among all chips to allow fair comparison. Two-step normalizations were used, including per-chip normalization (normalization to 50th percentile) and per-gene normalization from which the signal intensity in a given chip is divided by the average intensity of the same gene across all chips. According to their expression levels, a discrimination score of a given probe set was calculated based on: $R = (\text{PM} - \text{MM}) / (\text{PM} + \text{MM})$, while R is discrimination score, PM is perfect match, and MM is

mismatch. The distance between the discrimination score and the given discrimination threshold was tested using one-sided Wilcoxon signed rank test. Probe sets with P values lower than 0.04 were considered present (P); those with P values greater than 0.06 were absent (A); and those with P values between 0.04 and 0.06 were marginal (M). Furthermore, some microarray data as marked with $_x_$ at suffixes are not unique probe sets or are not identical probes among multiple transcripts. Therefore, we excluded all the A, M, and $_x_$ at probe data to increase the data accuracy and reliability. Fold change at 1.5 was used as a cutoff to filter experimental data compared with the control group. Then ANOVA with Dunnett adjustment was applied to assess gene expression difference between the treatment and the control groups. Finally, gene ontology (GO) Slims (<http://www.geneontology.org/GO.slims.shtml>) were applied to classify the differentially expressed genes into 21 GO categories based on the major biologic processes. The categories of TPA-induced cancer related genes, including tumor suppressor, apoptosis inducer, apoptosis inhibitor, and oncogene, were further presented.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) Confirmation. RT-PCR reaction was carried out using purified total RNA obtained as described above. The cDNA was synthesized by RT-PCR using a one-step RT-PCR kit (Qiagen). The primers are derived from published gene sequences as follows: MAPK1: sense primer, 5'-TCT CCC GCA CAA AAA TAA GG-3'; antisense primer, 5'-TCG TCC AAC TCC ATG TCA AA-3'; H-ras: sense primer, 5'-TGT TAC CAA CTG GGA CGA CA-3'; antisense primer, 5'-TCT CAG CTG TGG TGG TGA AG-3'; PI3Kca: sense primer, 5'-TGT TTG CAA AGA AGC TGT GG-3'; antisense primer, 5'-TAT GAC CCA GAG GGA TTT CG-3'; IGFBP3: sense primer, 5'-AAG TTC CAT CCA CTC CAT GC-3'; antisense primer, 5'-AGC TCT GCT TTC TGC CTT TG-3'; lepr: sense primer, 5'-AGG CCC AGA CAT TTT TCC TT-3'; antisense primer, 5'-TCC TGG AGG ATC CTG ATG TC-3'; β -actin: sense primer, 5'-TGT TAC CAA CTG GGA CGA CA-3'; antisense primer, 5'-TCT CAG CTG TGG TGG TGA AG-3'. PCR reaction (50 μ l) was run with a final concentration of 200 μ M dinucleotide triphosphate mix, 1 \times PCR buffer, 1 μ M of each primer, and 1.0 units Taq polymerase. Thermal cycling conditions, following an initial denaturation at 94°C for 4 mins, were as follows: 30 secs at 95°C, annealing at 55°C for 30 secs, and extension at 72°C for 1 min. Then, samples were incubated at 72°C for 7 mins. Amplified products at 8 μ l were loaded and separated on a 1.5% agarose gel. The RT-PCR products were visualized under ultraviolet light by the FluorChem 8800 Advanced Imaging System (Alpha Innotech, San Leandro, CA). The relative density of the target band was normalized to the loading control β -actin and then expressed as a percentage of the controls.

Statistic Analysis. The overall effects of treatments on body weight, fat composition, and RT-PCR expression

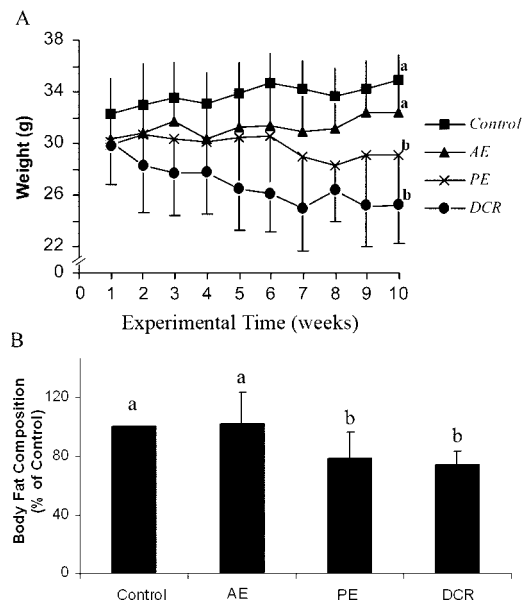


Figure 1. Effects of DCR and exercise treatment on body weight and fat composition. (A) Body weight. (B) Fat composition. Results are means \pm SE, $n = 8$ to 12. Means with different alphabetical letters differ significantly, $P \leq 0.05$.

levels were analyzed by one-way ANOVA, and then Dunnett method and least significant difference (LSD) were used to assess the differences between the treatment groups and the control group. The statistical significance of difference was set at $P \leq 0.05$.

Results

Impact on Body Weight and Body Fat Composition. Lean adult SENCAR mice in the control group gradually gained weight throughout the experimental period, whereas mice at 20% DCR or exercise with pair-feeding consistently lost weight. By the end of the experiment, the weights of the DCR and PE mice were significantly lower than those of the control mice (Fig. 1A). Exercise with *ad libitum* feeding (AE) did not lower weight significantly when compared with the sedentary counterparts, which might be, at least in part, due to the increased food intake (4.0 ± 0.2 g/day for the control mice vs. 4.3 ± 0.4 g/day for AE mice). Consequently, percent fat composition, as shown in Figure 1B, significantly decreased in the PE and DCR groups but not the AE group compared with the controls. No significant change of bone density was found among groups (data not shown).

TPA-Induced Gene Expression Profiles in Response to DCR and Exercise Treatments. We measured 45,101 gene probe sets expressed in the skin tissues of the DCR-fed or exercise-treated mice. We compared the distribution of the scatter plots among AE, PE, and DCR and found that DCR treatment produced the most extensive range of the gene expression levels. A condition tree by using hierarchical clustering method further displayed the similarity of gene profiles, as indicated

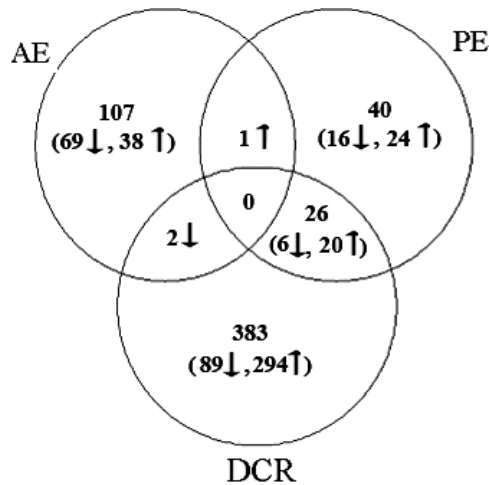


Figure 2. Venn diagram with number of genes altered in each group, shared across two groups, and shared across all three groups. Left: Genes that are 1.5-fold significantly downexpressed in comparison with the control. Right: Genes that are 1.5-fold significantly overexpressed in comparison with the control.

by a distance between two groups. The smaller the distance, the closer the gene expression profiling between two groups. Since the results showed a distance at 0.27 between DCR and PE, 0.69 between AE and the control, and 0.96 between DCR and AE, we were convinced that the gene expression pattern of PE group was relatively similar to that of DCR, whereas AE was closer to the control.

TPA-Induced Expression of Genes Regulated by DCR or Exercise Treatments. We identified 559

genes that showed at least 1.5-fold significant change ($P \leq 0.05$) by either DCR or exercise treatment compared with the control. As shown in Figure 2, we recognized that 411 genes (97 suppressed and 314 overexpressed) were altered by DCR, 110 genes (71 suppressed and 39 overexpressed) were changed by AE, and 67 genes (22 suppressed and 45 overexpressed) were regulated by PE. It should be noted that two genes relating to RIKEN cDNA (AK009351) and plasmalemma vesical-associated protein (NM032398) were downregulated in both the AE and DCR groups, and six genes, including RIKEN mRNA sequence (BB143476), MARCKS-like protein (NM010807), major urinary protein 3 (M27608), sortilin 1 (AV247637), endothelin receptor B (BB770914), and ATPase (BC001995), were downregulated in both the PE and DCR groups. It was interesting that only 1 gene corresponding to sequence AA407809 was overexpressed in both AE and PE groups, but 20 genes, such as casein kinase II (BG070990), forming binding protein 2 (BB817145), adenylylate cyclase 1 (AI848263), sperm-associated antigen 1 (NM012031), sialyltransferase 10 (NM018784), SH3 domain protein D19 (NM012059), cystathionine beta-synthase (BC026595), galactosylceramide sulfotransferase (AK002510), and proline dehydrogenase (NM011172), were overexpressed in both the PE and DCR groups.

Functional Overrepresentation Analysis. Functional overrepresentation analysis by GO annotation was performed to identify major biologic processes potentially impacted by the treatments. Figure 3 shows 21 GO categories that were significantly impacted by either calorie

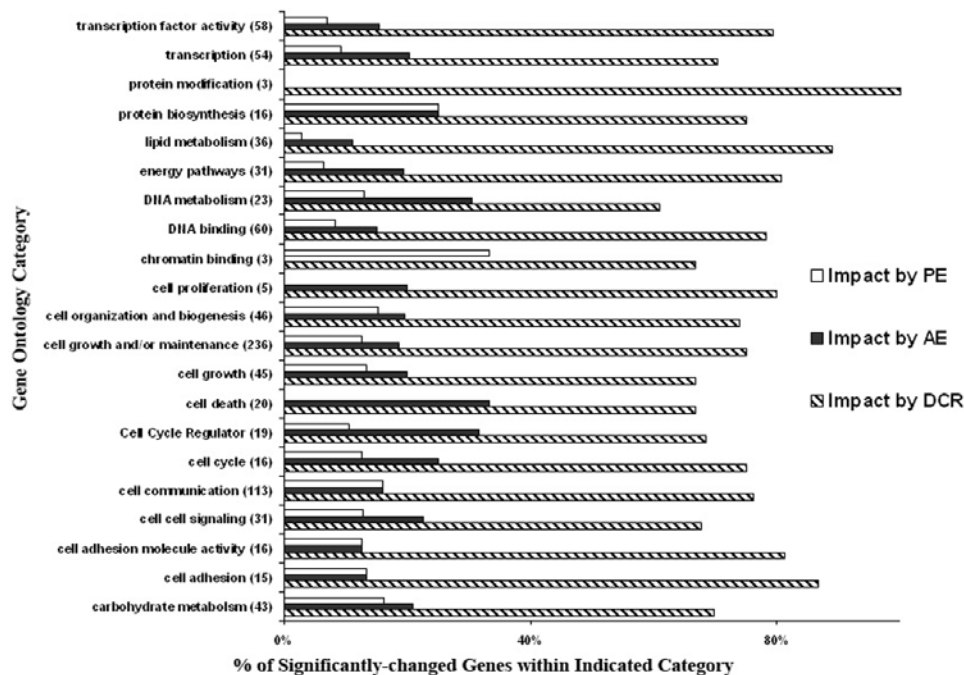


Figure 3. Functional overrepresentation analysis of gene expression altered by DCR and exercise treatments. GO Slims were used to show the percentage of TPA-induced gene expression altered by AE, PE, and DCR with a given GO category. Each gene category shown represents the combined genes that are significantly altered ($P < 0.05$) at greater than 1.5-fold change. The number of genes for each category is shown in parentheses.

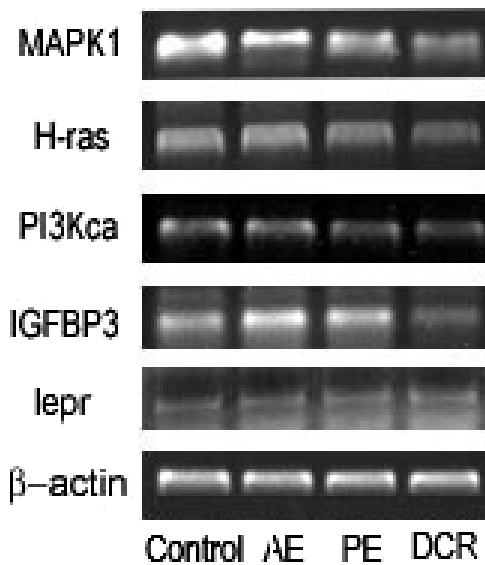


Figure 4. Confirmation of microarray data by RT-PCR. Five genes were randomly chosen from cancer-related categories, and their expression patterns in comparison with the microarray data were validated by RT-PCR. Identical results were obtained with MAPK1, PI3Kca, and IGFBP3 genes, which downexpressed significantly in DCR group. The expression of β -actin gene was used as a loading control.

restriction or exercise, along with the percentage of each treatment-induced gene expression frequency. Of the significantly changed genes, DCR showed considerable impact, accounting for ~80% compared with PE or AE, which usually were less than 20%. Specifically, most genes identified in our study are involved in cell growth/maintenance and cell communication categories, following by DNA binding, transcription factor activity, and transcription categories.

RT-PCR Confirmation. The microarray data were further validated using RT-PCR for five randomly selected genes in cancer-related categories. As shown in Figure 4, five representative gels showed the expression patterns of five genes in comparison, with β -actin as a loading control. Given these RT-PCR data, we found the gene expressions of MAPK1, PI3Kca, and IGFBP3 were significantly decreased in the DCR group compared with the control group. Both H-ras and lepr were not significantly changed between experimental groups. The RT-PCR confirmation rate to the microarray data set as estimated by using a Bayesian statistical method is about 93.3%.

Impact on TPA-Induced Cancer-Related Genes.

Apart from the genes relevant to nutrient metabolisms and other biologic processes, we further identified 22 genes related to TPA-induced cancer risk using the GO Slims. As shown in Table 1, we found that DCR induced overexpression of three tumor suppressor genes, four apoptosis inducers, and one apoptosis inhibitor. DCR also induced downexpression of two apoptosis inducers and eight oncogenes. In comparison with DCR, the impact of PE on expression of these genes is

moderate, with PE inducing downregulation of one apoptosis inducer gene and four oncogenes and upregulation of one apoptosis inducer gene. In contrast, AE was associated with downexpression of one apoptosis inducer gene and one oncogene but upexpression of one apoptosis inhibitor gene and one oncogene.

Discussion

Weight control can be achieved by either reducing energy intake, such as through DCR, or enhancing energy expenditure, such as through exercise. While 20% DCR reduced body weight significantly, corresponding to decreased fat composition, the treadmill exercise under this experimental condition demonstrated only a modest weight loss. Exercise alone with *ad libitum* feeding was not sufficient for decreasing body weight due, at least in part, to the corresponding increase in diet intake. If the food intake of the exercised mice was limited by pair-feeding with their sedentary counterparts, then body weight and fat composition were modestly but significantly reduced. It should be noted that the interaction of dietary and physical activity upon energy expenditure and homeostasis is complicated. In addition to dietary energy intake and physical activity, for example, the thermogenesis responsible for dietary change and the energy expenditure required for performance of cellular function also may play an interactive role in weight change (30).

Using our established strategies to control body weight in SENCAR mice, we measured the genomic gene expression in TPA-promoted skin tissues and compared the TPA-induced gene expression profiles among the AE, PE, and DCR treatments. Of the 39,000 transcripts with 45,101 probe sets measured, we identified 559 genes that showed at least 1.5-fold significant change by DCR and/or exercise treatments in comparison with the controls. The 1.5-fold significant change was selected because it showed a consistent result with a relatively large expression and conservative level (22, 31). However, the cutoff line at 1.5-fold change may exclude genes with <1.5-fold but significantly altered expression by the treatments.

Of these 559 genes, 411 genes (97 downexpression and 314 overexpression) were altered by DCR, 110 genes (71 downexpression and 39 overexpression) were changed by AE, and 67 genes (22 downexpression and 45 overexpression) were changed by PE. It is interesting to note the function of some specific genes that were co-regulated significantly by both the DCR and PE groups. For example, the gene encoding MARCKS (myristoylated alanine-rich C kinase substrate) protein was downexpressed significantly in both the PE and DCR groups but not the AE group. MARCKS protein is a widely distributed substrate for protein kinase C (32), and activation of protein kinase C has been well recognized as an initial signal in TPA-induced tumor promotion in mouse skin carcinogenesis (33). In addition, an upregulation of histone deacetylase 11 gene

Table 1. Identified TPA-Induced Cancer-Related Genes Altered by DCR, PE, and AE Treatments

Ontology category and probe	Gene name	Fold change (<i>P</i> value) ^a		
		DCR	PE	AE
Tumor suppressor				
1429418_at	RIKEN cDNA A530086E13 gene	+1.52 (<0.036)	—	—
1418146_a_at	Retinoblastoma-like 2	+1.61 (<0.030)	—	—
1454867_at	Mus musculus transcribed sequence with moderate similarity to protein sp:Q10571 (<i>Homo sapiens</i>) MN1_HUMAN Probable tumor suppressor protein MN1	+1.70 (<0.024)	—	—
Apoptosis inducer				
1449317_at	CASP8 and FADD-like apoptosis regulator	-2.91 (<0.002)	-1.62 (<0.002)	—
1418011_a_at	SH3-domain GRB2-like B1 (endophilin)	+1.52 (<0.029)	—	—
1450731_s_at	Tumor necrosis factor receptor superfamily, member 21	+1.96 (<0.026)	+1.80 (<0.026)	—
1433938_at	Transformation-related protein 53 binding protein 2	+1.72 (<0.025)	—	—
1449591_at	Caspase 4, apoptosis-related cysteine protease	+1.59 (<0.014)	—	-1.81 (<0.045)
1426184_a_at	Programmed cell death 6 interacting protein	-2.12 (<0.042)	—	—
Apoptosis inhibitor				
1449033_at	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	+2.31 (<0.031)	+1.98 (<0.031)	—
1435024_at	Nucleolar protein 3 (apoptosis repressor with CARD domain)	—	—	+1.58 (<0.035)
Oncogene				
1422687_at	Neuroblastoma ras oncogene	-1.98 (<0.034)	—	—
1451715_at	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)	—	-1.64 (<0.008)	—
1427783_at	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	-3.34 (<0.033)	-2.47 (<0.033)	-1.69 (<0.033)
1417155_at	Neuroblastoma myc-related oncogene 1	-3.31 (<0.008)	-1.93 (0.008)	—
1424332_at	Rab40c, member of RAS oncogene family	-2.64 (<0.010)	—	—
1417656_at	Myeloblastosis oncogene-like 2	-2.18 (<0.001)	-1.64 (<0.001)	—
1422087_at	Lung carcinoma myc-related oncogene 1	-2.02 (<0.007)	—	—
1450194_a_at	Myeloblastosis oncogene	-1.95 (<0.040)	—	+1.67 (<0.040)
1422119_at	RAB5B, member of RAS oncogene family	-1.88 (<0.035)	—	—
1448885_at	RAP2B, member of RAS oncogene family	-1.70 (<0.006)	—	—
1416591_at	RAB34, member of RAS oncogene family	-1.52 (<0.035)	—	—

^a The fold change is a ratio of the gene expression in the treatment group to the controls, which is denoted as increased (+) or decreased (-) if the treatment group is greater or less than the control, respectively. Only the data that are greater or less than 1.5-fold with a significant change ($P < 0.05$) are present ($n = 4$ per group).

(BC016208), one of the large family of sirtunis, which includes SIRT1, by the DCR group is in agreement with the published reports by Cohen *et al.* (34). Furthermore, SIRT1 has been found to be required for the increased physical activity that is typically observed in calorie-restricted mice (35).

When compared with DCR treatment, which provided a profound impact on gene expression, the number of gene expressions altered by PE and AE were moderate. However, the similarity of the gene expression patterns altered by PE and AE appears considerably different. The results by condition tree analysis indicate that the gene expression pattern altered by PE treatment was relatively closer to DCR, whereas AE was closer to the control. This suggests that moderate exercise alone without diet control may have

little effect on DCR-like gene expression patterns. The combination of aerobic exercise with diet control, however, not only lowered body weight but also provided a DCR-like impact on gene expression patterns. This observation is further supported by the results of GO annotation analysis.

Using GO annotation, we identified all the altered genes in 21 GO categories for major biologic processes. Specifically, we listed 22 genes that are classified into four TPA-induced, cancer-related categories. It is not unexpected that DCR provided an overwhelming impact on the expression of these cancer-related genes, generally in favor of cancer prevention through induction of overexpression of tumor suppressor and apoptosis inducer genes and down-expression of oncogenes. It is interesting that PE induced a moderate but similar impact to DCR on oncogene

expression, especially in downregulating the oncogenes. In contrast, AE showed the least impact on these cancer-related genes. Considering the impact on body weight and fat composition as well as the similarity of the gene expression patterns, we may deduce that PE could be a better alternative than AE to DCR-like cancer prevention via modification of gene expression patterns such as down-regulation of certain oncogenes.

Frankly speaking, it is challenging and ambitious to specify all the significantly changed genes and their potential roles accounting for cancer prevention. Although microarray analysis is useful for identifying potential gene expression and enhancing our understanding of cancer prevention by weight control, future studies incorporating gene expression data with proteomics may provide more insights.

Nevertheless, this pilot study reports the altered mRNA expression of certain genes in weight control mice via either reduced dietary energy intake or increased energy expenditure. DCR treatment provided substantial weight loss and significantly modified the gene expression profile. PE induced a modest impact on both weight loss and gene expression. In contrast to PE, AE was not associated with reduced body weight, and its effect on gene expression pattern was more similar to the controls. The data suggest that the degree of weight loss may be the critical indicator in reducing cancer risks.

We acknowledge the DXA analysis assistance of Dr. Mark Haub (Department of Human Nutrition, Kansas State University), the computational advice of Dr. Kesheng Liu (Department of Plant Pathology, Kansas State University), and the assistance in microarray data analysis from Mrs. Nanyan Weng (Gene Expression Facility, Kansas State University. This is journal contribution 04-429-J of the Kansas Agriculture Experiment Station, Manhattan, Kansas.

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