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Effects of a Black Raspberry Diet on Gene Expression in the Rat Esophagus

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A diet containing 5% freeze-dried black raspberries (BRB) markedly inhibits esophageal cancer in rats treated with the carcinogen, *N*-Nitrosomethylbenzylamine (NMBA). We previously identified esophageal genes that become dysregulated after short-term treatment of rats with NMBA and determined which genes are maintained at near-normal levels of expression if the animals were fed 5% BRB prior to and during NMBA treatment. In this study, we report the effects of the BRB diet on gene expression in esophagi from untreated (control) animals. After 3 wk on a 5% BRB diet, control esophagi were excised, stripped of the submucosal and muscularis layers, and processed for histology and microarray profiling. RNA microarrays revealed that the BRB altered the expression levels of 36 genes; 24 were upregulated, and 12 were downregulated. Among the upregulated genes are genes associated with cellular matrix, signaling cascades, transcription regulation, apoptosis, metabolism, and intriguingly, contraction. Most of the downregulated transcripts are involved in cell regulation, signal transduction, and metabolism. Histopathological analyses revealed that the BRB have little or no effect on esophageal morphology. In conclusion, histological and molecular studies indicate that a 5% BRB diet produces only modest effects on the esophagus, the target tissue for NMBA carcinogenesis in the rat.

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INTRODUCTION

We (1, 2) have shown that the induction, by *N*-Nitrosomethyl benzylamine (NMBA), of esophageal tumors in rats can be antagonized by providing the animals a diet supplemented with the naturally occurring compounds: phenethyl isothiocyanate (PEITC) and ellagic acid (EA). Berries are an abundant source of EA (630 to 1,500 μg/g dry weight); most of it is found in the pulp and seeds of the berry with very little in the juice (3). Because berries are 85–90% water, we elected to concentrate the EA in berries by eight- to ninefold through freeze drying. Further experimentation (4–8) has shown that a diet containing 5–10% freeze-dried berry powder effectively reduced the number of esophageal tumors (papillomas) in NMBA-treated rats by 40–60%. In addition, extensive histopathological studies of multiple tissues from rats fed a synthetic diet containing either 5% or 10% freeze-dried black raspberries (*Rubus occidentalis)* (BRB) or strawberries (*Fragaria ananasia*) for a period of 9 mo showed no evidence of toxic changes in any of the tissues (9). These results suggested that berries exhibit chemopreventive efficacy at concentrations in which they elicit little or no toxicity. Nevertheless, berries are known to contain several biologically active agents such as EA, ß-carotene, α-carotene, lutein, gallic acid, ferulic acid, *p*-coumaric acid, quercetin, ß-sitosterol, stigmasterol, anthocyanins, and kaempferol, many of which exhibit toxic effects in vitro (8, 10). Thus, we decided to use microarray techniques to determine whether a diet containing 5% freeze-dried BRB induces changes in the transcriptome of the rat esophagus without affecting tissue morphology. This

report summarizes the results of these studies and delineates those esophageal genes that showed changes in their transcription levels as a response to berry treatment.

MATERIALS AND METHODS

Animals, Diet and BRB

Male F344 rats, 4 to 5 wk old, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). The rats were housed and maintained under standard conditions (20 ± 2°C; 50 ± 10% relative humidity; 12-h light/dark cycle). AIN-76A synthetic diet was purchased from Dyets, Inc. (Bethlehem, PA). To insure that the diet containing 5% BRB was isocaloric with the control diet, the berry-containing diet was compounded by Dyets, Inc. to contain 5% less starch. Fresh frozen Jewel variety BRB were purchased from the Stokes Fruit Farm (Wilmington, OH) and shipped frozen to Van Drunen Farms (Momence, IL) where they were freeze dried and ground into a powder. The berry powder was shipped frozen to our laboratory and stored at –20°C until mixed into AIN-76A diet using a Hobart mixer (4–6). The resultant diet was stored at 4°C until fed to the animals. The animals were provided the diet and water ad libitum. All experimental protocols were in accordance with National Institute of Health guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University.

Experimental Protocol

Rats were randomized into 2 experimental groups of 9 animals each. Rats in Group 1 were placed on control AIN-76A diet and those in Group 2 were given AIN-76A diet plus 5% BRB. After 3 wk, the animals in both groups were sacrificed. Their excised esophagi were opened longitudinally and sectioned into 2 parts. One part was fixed for 24 h in buffered formalin, and the histopathology (hematoxylin and eosin) was evaluated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The other portion was stripped of the muscularis layers and immersed in 1 ml of TRIZOL® Reagent (Life Technologies, Gaithersburg, MD) for RNA extraction.

RNA Extraction and Microarray Analysis

RNAs were purified using a Qiagen RNeasy mini kit (Qiagen, Valencia, CA). The microarray analyses were carried-out using Agilent Rat Whole-Genome arrays (#G4131F, Agilent Technologies, Santa Clara, CA) with 60-mer oligonucleotide probes and over 41,000 transcripts as described before (11). Triplicate microarrays were completed for each group; each microarray was done using pooled RNA samples from 3 esophagi. Each microarray was hybridized against universal rat reference RNA purchased from Stratagene (La Jolla, CA). The hybridization reactions consisted of 0.75 μg of each preparation. They were cohybridized on the array for 17 h at 60°C and then washed successively with standard saline citrate (SSC), 0.005% Triton X-102 for 10 min at room temperature, and 0.1 × SSC, 0.005% Triton X-102 for 5 min on ice. Dried slides were immediately scanned using an Agilent G2565AA dual laser scanner.

Tiff images of the hybridized arrays were analyzed using feature extraction software (Agilent version 7.5) for fluorescent intensities after local background subtraction and Linear & Lowess normalization (12). Outliers and saturated features were excluded. Normalized data were imported into Rosetta Resolver (version 5.1.0.1.23; Rosetta Biosoftware, Kirkland, WA) for 1-way ANOVA analyses to identify statistically significant (P value $\leq$0.001) transcripts per treatment (13). The data cutoff was set at a minimum 1.5-fold change to minimize false positives. In addition, the data were confirmed by comparison with mRNA levels obtained by quantitative reverse transcription-polymerase chain reaction using selected gene-specific primer pairs (see our previous report) (11). The probe accession tags were converted to Entrez GeneIDs (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene) so the analyses could based on gene names. Differentially expressed genes were classified into functional groupings using the Gene Ontology Consortium designations and DAVID (http://david.abcc.nciferf.gov) (14,15).

RESULTS AND DISCUSSION

Effect of BRB on Morphology of Rat Esophagus

Examples of photomicrographs of esophageal tissues taken from 5 rats on control diet and 5 rats on the 5% BRB diet are shown in Fig. 1. In agreement with our previous studies (5, 6, 11), statistical evaluation of the slides revealed no significant differences (Student's t-test $>$ 0.01) in morphological appearance of esophagi between the 2 groups (Table 1).

Effect of BRB on Rat Esophagus Gene Transcription

We (11) previously reported that phenylethyl isothiocyanate (PEITC), a chemopreventive constituent of cruciferous vegetables, produced changes in the expression levels of 251 genes in F344 rat esophagus when fed in AIN-76A diet for a period of 3 wk. Because freeze-dried BRB contain numerous molecularly active chemopreventative compounds, for example, EA,

TABLE 1
Effect of BRB on histopathology of rat esophagus (see Materials and Methods for experimental details)[a]

	Histopathology Classification as Percentage (± SD)		
Treatment	Normal	Epithelial Hyperplasia	Dysplasia
Control AIN-76A diet	81 (10)	19 (10)	0
AIN-76A + 5% BRB diet	73 (9)	27 (8)	0

[a] Abbreviation is as follows: BRB, black raspberry.

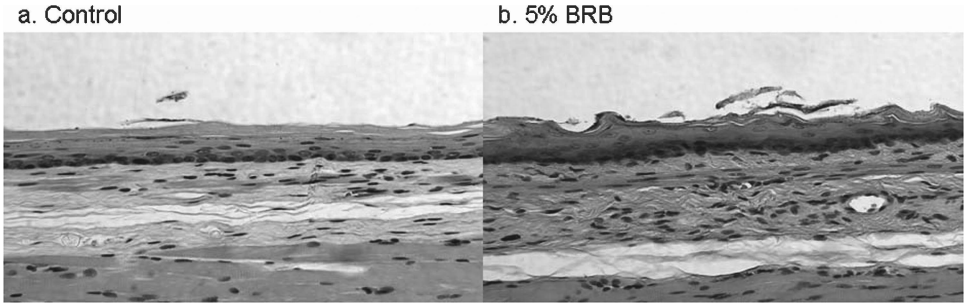

FIG. 1. Histological appearance of esophagi from rats fed AIN-76A control diet (a) and control diet plus 5% BRB (b). Tissue sections (magnification × 100) are stained with H&E.

ß-carotene, α-carotene, lutein, gallic acid, ferulic acid, *p*-coumaric acid, quercetin, ß-sitosterol, stigmasterol, anthocyanins, and kaempferol (6–10), we anticipated that the esophagi of rats consuming a berry diet would also exhibit multiple changes in their constellation of expressed genes. However, rats that consumed the 5% BRB diet for 3 wk exhibited differential expression of only 36 genes in their esophagi when compared to the esophagus of rats fed control AIN-76A diet (listed in Table 2). The reason(s) for the relative paucity of transcription changes in the esophagi of BRB-fed rats requires further investigation. However, 1 significant difference between the PEITC and BRB experiments is that PEITC was administered in the diet at a concentration (6 μmol/g) that inhibited NMBA-esophageal tumorigenesis nearly 100% (1), whereas the maximal level of inhibition elicited by 5% BRB was 48% (6). Thus, one might expect fewer changes in esophagus gene expression in rats fed PEITC at a concentration that reduces the tumor response by only 50%. On the other hand, that only 36 esophagus genes were affected supports our previous conclusion that a diet containing 5% freeze-dried BRB is not significantly toxic.

Of the differentially expressed genes, 24 showed increased transcription, and 12 were downregulated. Although the esophageal specimens were carefully stripped of the submucosal and muscularis layers before extracting their RNA, 10 of the 24 upregulated transcripts [myosin, heavy polypeptide 1; adducin 2 (beta); enolase 3, beta (Eno3); myosin, light polypeptide 2; actin, alpha cardiac muscle 1; tropomyosin 1, alpha (Tpm1); creatine kinase, mitochondrial 2; titin; actin, alpha 1; and acylphosphatase 2 (Acyp2 predicted)]. are associated with muscle and/or cell contraction processes. Further, the DAVID program clustered 6 of these genes (see bold font names in Table 2) as belonging to sarcomere function. Of these, 2, Acyp2 predicted and Eno3, are involved in the glycolytic metabolism of glyceraldehyde-3 phosphate to phosphoenolpyruvate. This elevated response may be due to the sugars within the freeze-dried BRB preparation. Overall, it was surprising to find so many muscle-related genes that were upregulated. Our interpretation of this observation is that the thin muscularis mucosae region, which is comprised of smooth muscle cells, lies directly beneath the stratified squamous epithelium of the esophagus (16), and it is plausible that some smooth muscle cells remained with the epithelium as it was separated from the submucosal and muscularis externa (stratified muscle) regions. It is unknown whether or how affecting the transcription of muscularis mucosae smooth muscle cells affects esophageal tumor development.

Interestingly, 2 of the upregulated genes, that is, rhesus blood group-associated C glycoprotein (Rhcg) and Tpm1, are commonly downregulated in esophageal tumor tissue. Rhcg is an ammonium transporter (17). The upregulation of Rhcg suggests that the BRB diet depresses ammonium balance in the esophagus, and excess Rhcg protein may be necessary to correct the balance. Alternatively, as Rhcg is most frequently expressed in squamous tissues, its upregulation may reflect an ability of BRB to augment squamous differentiation processes in the esophageal epithelium. If this is correct, one mechanism of chemoprevention by BRB is that of promoting terminal differentiation. In agreement with this hypothesis, our microarray studies showed that the BRB diet also increased transcription of filaggrin, a component of the cornified envelope required for terminal differentiation of esophageal epithelium (18). Another upregulated gene that is frequently downregulated in human esophageal squamous cell carcinomas (SCC) is Tpm1 (19,20). Tpm1 is a tumor suppressor gene involved in regulation of the cytoskeleton, and its suppression may contribute to metastatic properties of tumor cells. It has been shown that Tmp1 is downregulated by hypermethylation of the promoter region of the gene (21,22). It will, therefore, be interesting to determine if BRB affects the methylation density of the Tpm1 promoter and, if so, whether BRB stimulates hypomethylation or inhibits hypermethylation.

Antioxidant activity is a known anticancer mechanism of berries (23), and it was expected that transcription of oxidative damage genes should be changed in the esophagus of rats that consumed the BRB diet. One gene that was upregulated was RGD1564596 predicted, which is the rat homologue of the human glyoxalase domain containing 5. This enzyme catalyzes a generalized oxidation-reduction reaction wherein hydrogen ions or electrons are transferred from one donor, and 2 oxygen atoms are incorporated into another donor. The

TABLE 2
Transcripts upregulated and downregulated in rat esophagus by black raspberry diet

Accession	Gene ID	Official Symbol	Official Full Name, Function, and Comments	Fold Change
Upregulated Travnscripts				
XM 237966	290612	Sh2d4b predicted	SH2 domain containing 4B (predicted). Involved in intracellular signaling cascades.	6.70
AW921971	293048	Rhcg	Rhesus blood group-associated C glycoprotein. Facilitates rapid and low-energy-dependent bidirectional ammonium movement across the plasma membrane, and with RhBG and RhAG constitute a family of NH_3 channel proteins in mammalian cells. Expression is frequently lost/reduced in esophageal cancer tissue.	5.18
XM_213345	287408	Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult. Control of fiber shortening speed and resistance to fatigue. Cytoskeleton organization and muscle development.	5.06
XM_216406	298077	RGD1305807	Hypothetical LOC298077. Function unknown	4.80
CB544782	497815	Nrcam	Neuron-glia-CAM-related cell adhesion molecule. Ankyrin-binding protein. A target gene of β-catenin signaling with a role of vascular tube formation and angiogenesis.	4.26
X59736.1	117001	Ckmt2	Creatine kinase, mitochondrial 2, sarcomeric. Catalyzes the transfer of high energy phosphate to creatine in tissues with large, fluctuating energy demands, such as skeletal muscle, heart, and brain.	4.12
XM_342715	24171	Add2	Adducin 2 (beta). Membrane skeletal protein involved at sites of cell-cell contact in epithelial tissues. Expressed in endothelial, vascular smooth muscle, and kidney tubular cells. Role in ion transport.	3.83
BF397107	297867	Arid1a predicted	AT rich interactive domain 1A (Swi1 like) (predicted). Member of the SWI/SNF family, with helicase and ATPase activities. Thought to regulate transcription by altering the chromatin structure around genes; it is critical for tumor suppression.	3.67
NM_012949	25438	Eno3	Enolase 3, beta. In adult skeletal muscle cell; involved in striated muscle development and regeneration.	3.66
NM_012605	17906	Myl2	Myosin, light polypeptide 2, regulatory, cardiac, slow. Myosin regulatory light chain 2, ventricular/ cardiac muscle isoform. Ca+ triggers the phosphorylation of regulatory light chain that in turn triggers contraction.	3.45
AA926062	29139	Dcn	Decorin. Cellular or pericellular proteoglycan; binds to the N-terminal region of collagen VI; has role in matrix assembly.	3.44

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TABLE 2
Transcripts upregulated and downregulated in rat esophagus by black raspberry diet *(Continued)*

Accession	Gene ID	Official Symbol	Official Full Name, Function, and Comments	Fold Change
XM_217593	302554	RGD1564596 predicted	Similar to RIKEN cDNA 2010001H14 (predicted). Human/mouse homologue is glyoxalase domain containing 5 (GLOD5).This protein catalyses an oxidation-reduction (redox) reaction. Catalysis of the reaction: biphenyl-2,3-diol + O_2 = 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate + H_2O.	3.28
XM_215801	29275	Actc1	Actin alpha cardiac 1. A structural component of the cytoskeleton; alpha actins are found in muscle tissues and is a major constituent of the contractile apparatus.	3.21
NM_019131	24851	Tpm1	Tropomyosin 1, alpha. Actin-binding protein of the contractile system of striated and smooth muscles, and stabilizes cytoskeleton actin filaments in nonmuscle cells. Downregulated in esophageal cancer tissue.	2.88
AW918824	84015	Ttn	Titin. Abundant in striated muscle; sarcomere organizer and is necessary for sarcomerogenesis. Adhesion template for the assembly of contractile machinery in muscle cells. Identified role in chromosome condensation and segregation.	2.56
NM_019212	29437	Acta1	Actin, alpha 1, skeletal muscle. Expressed in skeletal muscle; major constituent of the contractile apparatus. Structural component of the cytoskeleton; involved in cell motility.	2.47
XM_344275	364225	Acyp2 predicted	Acylphosphatase 2, muscle type similar to macrophage migration inhibitory factor (predicted). Hydrolyzes the phosphoenzyme intermediate of Ca2+/Mg2+-ATPase membrane pumps from sarcoplasmic reticulum of skeletal muscle.	1.97
BE107309	300289	RGD1564027 predicted	Similar to angiomotin (predicted). An angiostatin binding protein expressed predominantly in endothelial cells. May promote neoplastic angiogenesis by stimulating invasion and tube formation, and stabilizing established tubes. Role in the assembly of endothelial cell-cell junctions.	1.92
XM_345674	366624	Cfl2 predicted	Cofilin 2, muscle (predicted). Actin-binding protein; controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. It has the ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. Major component of intranuclear and cytoplasmic actin rods.	1.92
NM_031831	83765	Rtn4	Reticulon 4. Associated with endoplasmic reticulum. Interacts with Bcl-xl and Bcl-2 and reduces their anti-apoptotic activity.	1.72
LOC302332	24942	Chm	Choroidermia. May function as Rab escort protein.	1.57

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TABLE 2
Transcripts upregulated and downregulated in rat esophagus by black raspberry diet *(Continued)*

Accession	Gene ID	Official Symbol	Official Full Name, Function, and Comments	Fold Change
XM_238235	24641	Flg	Filaggrin. Major component of the keratohyalin granules of epidermis. Aggregates keratin intermediate filaments and promotes disulfide-bond formation among the intermediate filaments during terminal differentiation. A key regulator of terminal epidermal differentiation.	1.57
TC515147	500120	RGD1564384 predicted	Similar to serine/threonine kinase 31 (predicted). Encodes a putative protein kinase.	1.54
CB814173	296762	Phtf2 predicted	Putative homeodomain transcription factor 2 (predicted). Function unknown.	1.54
Downregulated Transcripts				
BE117471	308968	Rbbp6	Retinoblastoma binding protein 6. Protein binds to under-phosphorylated but not phosphorylated pRB. Involved in regulation of cell cycle. Involved in protein ubiquitination.	−1.50
XM_214152	289993	Cdkn3 predicted	Cyclin-dependent kinase inhibitor 3 (predicted). Dephosphorylates CDK2 kinase & thus prevents activation of CDK2 kinase. May play a role in cell cycle regulation. Interacts with CDC2, CDK2 and CDK3. Deleted, mutated, or overexpressed in several kinds of cancers.	−1.51
XM_345576	366461	RGD1564921 predicted Also called Cyclophilin H	Similar to peptidyl prolyl isomerase H (predicted). Accelerates folding of proteins and a protein chaperone that mediates the interactions between different proteins inside the spliceosome.	−1.52
XM_344506	364557	Anxa10	Annexin A10 (predicted). Calcium-dependent phospholipid-binding protein family member; may have role in regulation of cellular growth and in signal transduction pathways.	−1.53
TC489369	303132	Aff4 predicted	AF4/FMR2 family, member 4 (predicted). Positive regulator of Pol II transcription elongation factor b (P-TEFb) kinase; transcription factor. Component of (CDK9/cyclin-T1) complex.	−1.53
XM_232809	313087	Cpne3 predicted	Copine III (predicted). Calcium-dependent membrane-binding protein that may regulate molecular events. Exhibits calcium-dependent phospholipid binding properties.	−1.66
XM_227169	310436	RGD1564207 predicted	Similar to SET domain-containing protein (predicted). This record was discontinued; corresponds to unknown Genescan predicted transcript. Function unknown.	−1.76

(Continued on next page)

TABLE 2
Transcripts upregulated and downregulated in rat esophagus by black raspberry diet *(Continued)*

Accession	Gene ID	Official Symbol	Official Full Name, Function, and Comments	Fold Change
AI639014	297418	Gkn1	Gastrokine 1. Growth factor. Downregulated in human gastric cancer. Acts on specific tight junction proteins and stabilizing perijunctional actin. Has mitogenic activity and may be involved in maintaining the integrity of the gastric mucosal epithelium.	−4.72
XM_216206	297419	RGD1311934 predicted	Similar to RIKEN cDNA 1810036H07 (predicted) [Rattus norvegicus]. Mouse/human homologue of GKN2 (gastrokine 2). Binding partner of TFF1 in normal gastric mucosa.	−5.01
AW521721	24418	Grm5	Glutamate receptor, metabotropic 5. Glutamate receptor; activity mediated by G-protein that activates a phosphatidylinositol-calcium 2nd messenger system and generates a calcium-activated chloride current.	−5.26
BE106909	29338	Prdx2	Peroxiredoxin 2. Thioredoxin-dependent peroxide reductase; protective against oxidative damage by reactive sulfur species. Participates in signaling cascades of growth factors by regulating intracellular concentrations of H_2O_2. May play a role in cancer development.	−9.57
NM_181440	192266	Grpca	Glutamine/glutamic acid-rich protein A. Member of a family of contiguous repeat polypeptides that are related to proline-rich proteins but contain little proline. Function unknown.	−11.79

enzyme also catalyzes the addition of O_2 to biphenyl-2, 3-diol to produce 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate + water (H_2O; http://www.geneontology.org/index.shtml). The other BRB-affected oxidative damage gene, peroxiredoxin 2 (Prdx2), was surprisingly downregulated almost 10-fold by the 5% BRB diet. This gene is often upregulated in human esophageal cancer (24), and it also participates in signaling cascades of growth factors by regulating intracellular concentrations of hydrogen peroxide. Prdx2 may play another role in cancer development in addition to its antioxidant activity, as high levels of the protein inhibit apoptosis (25). Another apoptosis associated gene was reticulon 4 (Rtn4). Rtn4 interacts with basal cell lymphoma (Bcl)-extra large (xl) and Bcl-2 and reduces their antiapoptotic activity (26). Thus, the observed BRB-caused changes in both genes might be expected to increase apoptosis (although not to the degree that it could be detected by histopathology evaluation).

Two of the upregulated genes, RGD1564027 predicted and neuron-glia-CAM-related cell adhesion molecule (Nrcam), are primarily expressed by endothelial cells (27). RGD1564027 predicted is the human homologue of Amot, which codes for the angiostatin binding protein, angiomotin. Angiomotin is involved in the assembly of endothelial cell–cell junctions (28). Nrcam, a target gene of β-catenin signaling, is an ankyrin-binding protein involved in vascular tube formation and angiogenesis (27). The observation that the BRB diet upregulates these genes suggests that BRB promotes angiogenesis. This appears to contradict our previous observations in which esophageal tumors in NMBA-treated rats fed a 5% BRB diet were found to have a lower vascular density than tumors in companion rats treated with NMBA only (29). Thus, one might expect that BRB would downregulate RGD1564027 predicted and Nrcam because these genes promote angiogenesis. However, the expression of genes in tumor endothelium has been shown to vary from that in normal endothelium (30); thus, the downregulation of RGD1564027 predicted and Nrcam by BRB might not be unexpected.

Of the remaining upregulated genes, 4 are associated with cell architecture and matrix (decorin and cofilin 2, muscle predicted), signaling cascade (SH2 domain containing 4B-predicted), and in transcription regulation (adenosine triphosphate-rich interactive domain 1A-predicted); the remaining 5 transcripts are either not ascribed to known genes and/or are genes with no known function.

Regarding the 12 downregulated transcripts, most [retinoblastoma binding protein 6 (Rbbp6); RGD1564921 predicted; AF4/FMR2 family, member 4 predicted; copine III predicted, gastrokine 1, and) are involved in cell regulation and signal transduction. Interestingly, 2 of the downregulated transcripts, Rbbp6, and RGD1564921 predicted, were similarly affected by both BRB- and PEITC-alone diets (11), and both BRB and PEITC caused their downregulation. Rbbp6 encodes a 250-kDa ring finger-containing protein that is frequently upregulated in human esophageal SCC (31). The protein binds to underphosphorylated but not phosphorylated retinoblastoma protein (Rb). The phosphorylated Rb gene product binds the nuclear transcription factor E2F and prevents its ability to function in the S phase of the cell cycle (32). Thus, Rbpp6 competes with E2F for binding to the underphosphorylated form of Rb, and high concentrations of Rbpp6 protein, as are found in human esophageal SCC cells, would free E2F to stimulate cell proliferation. Rbbp6 also binds to p53, thereby enhancing Mdm2-mediated ubiquitination and degradation of p53 leading to decreased apoptosis (32, 33). Consequently, downregulation of Rbbp6 by BRB or PEITC should slow cell growth and increase the rate of apoptosis. The other common gene, RGD1564921 predicted (also called CypH), is a member of the cyclophilin family of proteins that transiently bind to other proteins and facilitates their folding. It may also serve as a bridge to mediate interactions between protein 60K of the U4/U6 small nuclear ribonucleoprotein and other factors (34). It is unclear how downregulation of RGD1564921 predicted might antagonize carcinogenesis.

CONCLUDING REMARKS

In summary, the transformation of a normal cell into a tumorigenic cell is driven by numerous molecular aberrations that endow them with resistance to apoptosis, self-sufficiency to growth signals, insensitivity to growth-inhibitory signals, limitless replicative potential, sustained angiogenesis, and tissue invasion/metastasis capabilities (35). Interestingly, a food preparation with excellent chemopreventive properties, BRB, is relatively innocuous with respect to its effects on histopathology and gene transcription. The BRB diet affected transcription of only 36 genes. Among these 36 were some previously associated with various cancer mechanisms, including oxidative damage, cell differentiation, cell communication/junctions, cytoskeleton, proliferation, and apoptosis. However, the importance of many of the 36 genes as antagonists of cancer remains to be delineated.

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