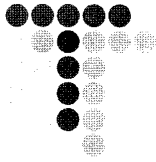

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# Analysis of Gene Expression Modulated by Indole-3-carbinol in Dimethylbenz[*a*]anthracene-induced Rat Mammary Carcinogenesis

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## Abstract

**Our previous finding that pre-initiation treatment of indole-3-carbinol (I3C) represents a chemopreventive effect in dimethylbenz[*a*]anthracene (DMBA)-induced mammary carcinogenesis has prompted us to test the global expression of genes at an early stage. Rats were continuously fed 300 ppm I3C in their diet at 6 weeks of age and were injected with DMBA at 7 weeks of age, and were sacrificed at 8 weeks of age. Global gene expression analysis using oligonucleotide microarrays was conducted to detect altered genes in DMBA- or DMBA plus I3C-treated mammary glands. Altered genes were identified by fold changes of 1.2 and by t-test ($P<0.05$) from the log ratios of the hybridization intensity of samples between control (Group 1) and DMBA (Group 2), and from those of samples between DMBA (Group 2) and DMBA plus I3C (Group 3). From these genes, we chose altered genes that were up- or down-regulated by DMBA treatment and recovered to the control level by I3C treatment. For early stage of carcinogenesis, I3C treatment induced the recovery to normal levels of several genes including cell cycle pathway (cyclin B2, cell division cycle 2 homolog A), MAP signaling pathway (fibroblast growth factor receptor 1, platelet derived growth factor receptor, beta polypeptide), and insulin signaling (protein phosphatase 1, regulatory (inhibitor) subunit 3B and flotillin 2), which were up-regulated by DMBA treatment. In addition, I3C treatment induced the recovery to normal levels of several genes including those of MAPK signaling (transforming growth factor, beta receptor 1 and protein phosphatase 3, catalytic subunit, beta isoform), which were down-regulated by DMBA treatment. These results suggest that the targeting of these genes presents a possible approach for chemoprevention in DMBA-induced mammary carcinogenesis.**

**Keywords:** Dimethylbenz[*a*]anthracene (DMBA), Microarray, Rat, Mammary gland

Daily contact with some environmental carcinogens is virtually unavoidable, and this is closely associated with the occurrence of cancer[1,2]. Primary prevention of cancer means preventing the occurrence of cancer by removing causes or providing inhibitory materials. Secondary prevention involves reducing mortality by finding cancer at an earlier stage. To achieve primary prevention of cancer, the application of several naturally occurring compounds from fruits and vegetables has many advantages[3], and the consumption of cruciferous vegetables such as cabbages and broccoli has been associated with cancer chemopreventive effects in humans[4].

Indole-3-carbinol (I3C), a component of cruciferous vegetables, has been suggested to have chemopreventive potential in experiments with animals and humans. I3C is produced endogenously from naturally occurring glucosinolates found in a wide variety of plant food substances, when they are crushed or cooked, and the acid environment of the gut easily converts them into a range of polyaromatic indolic compounds[5].

It has been reported that I3C has considerable potential as a natural prophylactic anticancer agent against some neoplasms such as in liver[6], colon[7] and uterus[8] when it is applied prior to or concurrently with exposure to the carcinogen. In the mammary gland, I3C treatment inhibited chemically-induced mammary tumors induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) in a rodent model[9,10], as well as naturally occurring mammary gland tumors[11].

This chemopreventive effect has been associated with the induction of phase I enzymes such as CYP1A1

and CYP1A2[12,13], and phase II enzymes such as glutathione *S*-transferase, NAD(P)H: quinone oxidoreductase, glutathione reductase, glutathione *S*-hydrolase[14,15], increased 2-hydroxylation in estrogen metabolism[16], pathways of cell cycle arrest[17,18], apoptosis of tumor cells[19], and the inhibition of cancer cell metastasis[20].

In our previous study, we found that post-initiation treatment of I3C did not inhibit either *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis[21] and another study found that I3C did not suppress or DMBA-induced one[22]. In other organ models, post-initiation treatment of I3C did not inhibit tumor formation, and it promoted tumor multiplicities in some cases[23,24].

As I3C and its several metabolites act to induce aryl hydrocarbon receptor mediating response and have an estrogenic effect[16,25], they may also have a carcinogenic effect in some situations, especially when initiated cells or tumor cells are growing. These findings have led us to consider time points of carcinogen exposure and the types of carcinogens and cellular circumstances when chemicals are applied.

It is reasonable that I3C induces the removal of a carcinogen before initiation. However, it is not clear which pathway(s) is affected by pre-initiation treatment with I3C. To gain a better understanding of the chemopreventive mechanisms of I3C in mammary carcinogenesis, we performed oligonucleotide microarray analyses using mammary glands of rats treated with I3C or normal diet and DMBA as a carcinogen, and compared the global gene alterations of I3C-treated mammary glands with those of DMBA-treated ones.

## Microarray Analysis

A hierarchical analysis revealed that each group was well classified and there was more similarity between the patterns of Groups 1 and 3 than between those of Groups 2 and 3 (Figure 1).

We extracted 179 genes showing a significant increase by comparison between Groups 1 and 2, yet no difference by comparison between Groups 2 and 3 (i.e. recovery to control level); 33 of selected genes had biological relevance to the KEGG pathway (Table 1). These included cell cycle-related genes such as cyclin B2 (Ccnb2) and cell division cycle 2 homolog A (Cdc2a); MAPK signaling-related genes such as fibroblast growth factor receptor 1 (Fgfr1), platelet derived growth factor receptor, beta polypeptide (Pdgfrb) and protein phosphatase 5 catalytic subunit (Ppp5c); insulin signaling-related genes such as protein phosphatase 1, regulatory (inhibitor) subunit 3B (Ppp1r3b) and flotillin 2 (Flot2).

In addition, we extracted 83 genes displaying a sig-

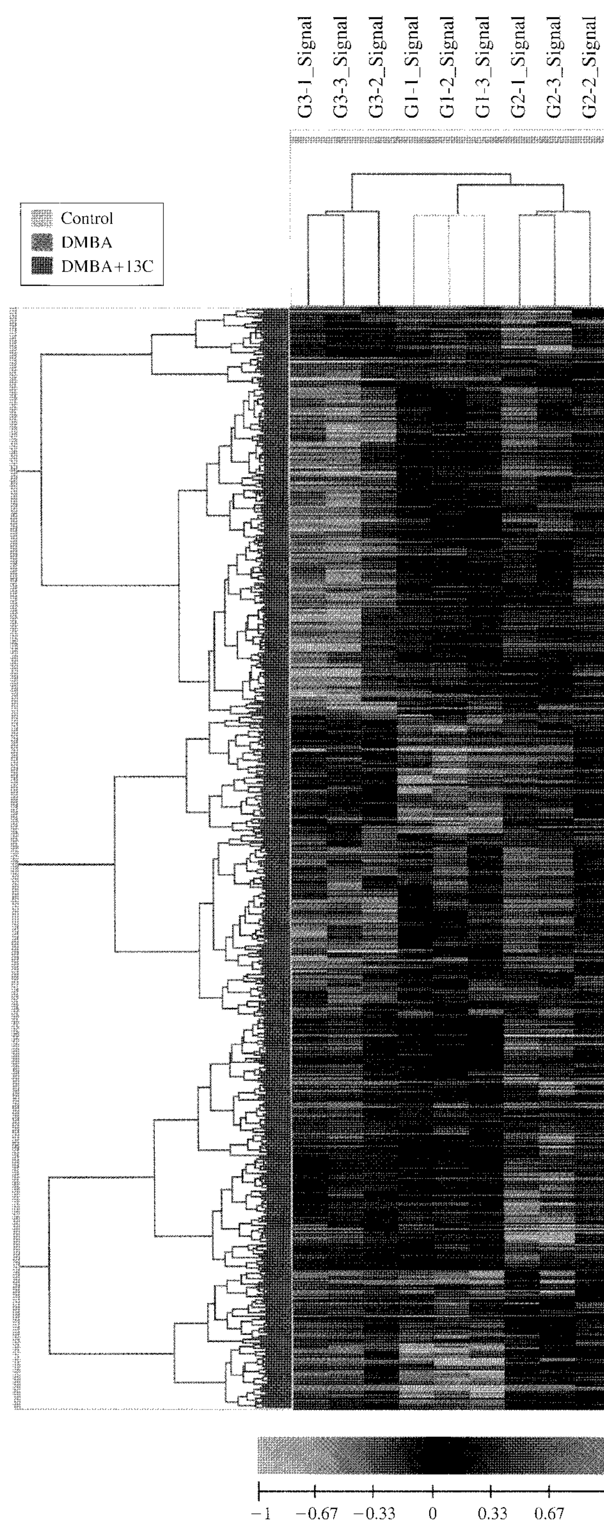


**Figure 1.** Gene expression in mammary gland from F344 rats treated with DMBA and/or I3C 300 ppm. Three categories of control, DMBA and DMBA plus I3C were classified, and analyzed. Clustering analysis of genes induced by control, DMBA and DMBA plus I3C, showing clear classification of three groups.

**Table 1.** Gene analysis from down-regulated genes by I3C treatment.

| Gene symbol | Gene title | RefSeq Transcript ID | Fold change G1 vs G4 | Fold change G1 vs G5 | KEGG_Definition |
|---|---|---|---|---|---|
| Itgb1 | integrin beta 1 (fibronectin receptor beta) | NM_017022 | 1.423767262 | 0.904607586 | Axon guidance; Focal adhesion; ECM-receptor interaction; Cell adhesion molecules (CAMs); Leukocyte transendothelial migration; Regulation of actin cytoskeleton; Small cell lung cancer |
| Esam | endothelial cell adhesion molecule | NM_001004245 | 1.653743325 | 0.905095962 | Cell adhesion molecules (CAMs); Leukocyte transendothelial migration |
| Lmna | lamin A | NM_001002016 | 1.616442662 | 1.12254556 | Cell Communication |
| Ccnb2 | cyclin B2 | NM_001009470 | 1.531405666 | 0.842172108 | Cell cycle; p53 signaling pathway |
| Cdc2a | cell division cycle 2 homolog A (S. pombe) | NM_019296 | 1.883328503 | 0.972959955 | Cell cycle; p53 signaling pathway; Gap junction |
| Sdhb_predicted | succinate dehydrogenase complex, subunit B, iron sulfur (Ip) (predicted) | XM_001071846 /// XM_216558 | 1.383089324 | 0.896158092 | Citrate cycle (TCA cycle); Oxidative phosphorylation |
| Sdhc | succinate dehydrogenase complex, subunit C, integral membrane protein | NM_001005534 | 1.542282627 | 0.691292095 | Citrate cycle (TCA cycle); Oxidative phosphorylation |
| Sord | sorbitol dehydrogenase | NM_017052 | 1.305630803 | 0.717887177 | Fructose and mannose metabolism |
| Gls | glutaminase | NM_001109968 /// NM_012569 | 1.443925125 | 0.95289679 | Glutamate metabolism; D-Glutamine and D-glutamate metabolism; Nitrogen metabolism |
| Mgll | Monoglyceride lipase | NM_138502 | 2.467841377 | 1.068977435 | Glycerolipid metabolism |
| Pigt_predicted | phosphatidylinositol glycan, class T (predicted) | NM_001106540 /// XM_001070371 /// XM_215919 | 1.497070332 | 0.904013987 | Glycosylphosphatidylinositol (GPI)-anchor; Glycan structures-biosynthesis 2 |
| Bmp7 | bone morphogenetic protein 7 | XM_001053727 /// XM_342591 | 1.73674282 | 0.943554856 | Hedgehog signaling pathway; TGF-beta signaling pathway |
| Hs6st1_predicted | heparan sulfate 6-O-sulfotransferase 1 (predicted) | XM_001108210 /// XM_001056379 /// XM_237060 | 1.22036299 | 0.851871069 | Heparan sulfate biosynthesis; Glycan structures-biosynthesis 1 |
| Ppp1r3b | protein phosphatase 1, regulatory (inhibitor) subunit 3B | NM_138912 | 2.676959168 | 1.382218155 | Insulin signaling pathway |
| Flot2 | flotillin 2 | NM_031830 | 1.236616077 | 0.830879979 | Insulin signaling pathway |
| Ppp5c | protein phosphatase 5, catalytic subunit | NM_031729 | 1.665770521 | 0.813983619 | MAPK signaling pathway |
| Fgfr1 | Fibroblast growth factor receptor 1 | NM_024146 | 1.333484141 | 0.983644108 | MAPK signaling pathway; Adherens junction; Regulation of actin cytoskeleton; Prostate cancer; Melanoma |
| Pdgfrb | platelet derived growth factor receptor, beta polypeptide | NM_031525 | 1.597176777 | 1.01302539 | MAPK signaling pathway; Calcium signaling pathway; Focal adhesion; Gap junction; Regulation of actin cytoskeleton; Colorectal cancer; Glioma; Prostate cancer; Melanoma |
| Ephx1 | epoxide hydrolase 1, microsomal | NM_001034090 /// NM_012844 | 1.212957639 | 0.727577668 | Metabolism of xenobiotics by cytochrome P450 |
| Ghrhr | growth hormone releasing hormone receptor | NM_012850 | 2.664307623 | 1.834264471 | Neuroactive ligand-receptor interaction |
| App | amyloid beta (A4) precursor protein | NM_019288 | 1.456437373 | 0.962542624 | Neurodegenerative Disorders; Alzheimer's disease |
| Psen2 | presenilin 2 | NM_031087 | 1.557210776 | 0.758243204 | Neurodegenerative Disorders; Notch signaling pathway; Alzheimer's disease |
| Man2c1 | mannosidase, alpha, class 2C, member 1 | NM_139256 | 1.676106514 | 1.058965068 | N-Glycan degradation; Glycan structures-degradation |

**Table 1.** Continued.

| Gene symbol | Gene title | RefSeq Transcript ID | Fold change G1 vs G4 | Fold change G1 vs G5 | KEGG_Definition |
|---|---|---|---|---|---|
| Rfng | Radical fringe gene homolog (Drosophila) | NM_021849 | 1.895552393 | 0.845579829 | Notch signaling pathway |
| Atp5d | ATP synthase, $H^+$ transporting, mitochondrial F1 complex, delta subunit | NM_139106 | 1.364155715 | 0.987882253 | Oxidative phosphorylation |
| Hmox2 | heme oxygenase (decycling) 2 | NM_024387 | 1.207129677 | 0.856866091 | Porphyrin and chlorophyll metabolism |
| Sec61a1 | Sec61 alpha 1 subunit (S. cerevisiae) | NM_199256 | 1.422523021 | 0.826330601 | Protein export |
| Ppp1r12b_predicted | Protein phosphatase 1, regulatory (inhibitor) subunit 12B (predicted) | NM_001107178 /// XM_001062402 /// XM_222656 | 1.514661346 | 1.006185822 | Regulation of actin cytoskeleton |
| Gabarap | gamma-aminobutyric acid receptor associated protein | NM_172036 | 1.246270728 | 0.926827978 | Regulation of autophagy |
| Stx5a | syntaxin 5a | NM_031704 | 1.347428762 | 0.938503805 | SNARE interactions in vesicular transport |
| Rab13 | RAB13, member RAS oncogene family | NM_031092 | 1.616463875 | 0.900301066 | Tight junction |
| Epb4.1l1 | erythrocyte protein band 4.1-like 1 | NM_021681 /// NM_172090 | 2.321635953 | 1.375053451 | Tight junction |
| Sh3gl1 | SH3-domain GRB2-like 1 | NM_031239 | 1.932571423 | 0.654449616 | Tyrosine metabolism; Phenylalanine metabolism; Glycerophospholipid metabolism; 1- and 2-Methylnaphthalene degradation; Benzoate degradation via CoA ligation; Limonene and pinene degradation; Alkaloid biosynthesis II |

nificant decrease by comparison between Groups 1 and 2, yet no difference by comparison between Groups 2 and 3 (i.e. recovery to control level), and selected 7 genes having biological relevance to the KEGG pathway (Table 2). These included MAPK signaling-related genes such as transforming growth factor, beta receptor 1 (Tgfbr1), and protein phosphatase 3, catalytic subunit, beta isoform (Ppp3cb).

In the histopathological examination of the mammary glands, morphological differences were not observed between the control and treated groups (data not shown).

## Discussion

In this study, we investigated the chemopreventive effect of I3C in DMBA-induced mammary carcinogenesis at an early stage using a microarray technique. We found that I3C treatment induced the recovery of altered genes induced by DMBA to control levels, including genes of cell cycle, MAPK signaling pathway, insulin signaling pathway, and TGF-beta pathway.

The microarray technique can provide a powerful tool to analyze many genes in a shorter time and to access a broad range of carcinogenesis research[26,27] and to obtain profiles induced by several chemicals[28-30].

As the microarray analysis of rainbow trout treated with I3C revealed increases in genes related to estrogen metabolism[31], I3C itself may exert estrogenic action in the mammary gland. It is known that estrogen or its metabolites can promote mammary tumors[32]; however, the treatment can inhibit mammary carcinogenesis, depending on the treatment time[33]. These data suggest that it is important to remind the cellular environment when chemicals are applied[34].

Even though treatment with I3C showed chemopreventive effects when it was applied prior to or simultaneously with carcinogen exposure[24], it was not effective when applied after carcinogen exposure[23]. Based on the finding that 13C had no effect on the rate of removal of DNA adducts when it was incorporated into the diet after carcinogen exposure[35], it would appear that it is important to repair the damage induced by carcinogens at an early stage.

In this study, I3C treatment inhibited cell cycle-related genes that were up-regulated by DMBA treatment. It has been reported that I3C treatment induces G1 arrest in a breast cancer cell line[17], and combination therapy with tamoxifen induces suppression of the growth of human breast cancer cells[18]. In this study,

**Table 2.** Gene analysis from up-regulated genes by I3C treatment.

| Gene symbol | Gene title | RefSeq Transcript ID | Fold change G1 vs G4 | Fold change G1 vs G5 | KEGG_Definition |
|---|---|---|---|---|---|
| Ppp3cb | protein phosphatase 3, catalytic subunit, beta isoform | NM_017042 | −1.24149791 | −0.957642996 | MAPK signaling pathway; Calcium signaling pathway; Apoptosis; Wnt signaling pathway; Axon guidance; VEGF signaling pathway; Natural killer cell mediated cytotoxicity; T cell receptor signaling pathway; B cell receptor signaling pathway; Long-term potentiation |
| Tgfbr1 | transforming growth factor, beta receptor 1 | NM_012775 | −1.429215699 | −1.06411593 | MAPK signaling pathway; Cytokine-cytokine receptor interaction; TGF-beta signaling pathway; Adherens junction; Colorectal cancer; Pancreatic cancer; Chronic myeloid leukemia |
| Crebbp | CREB binding protein | NM_133381 | −1.237478963 | −0.907236654 | Neurodegenerative Disorders; Cell cycle; Wnt signaling pathway; Notch signaling pathway; TGF-beta signaling pathway; Adherens junction; Jak-STAT signaling pathway; Long-term potentiation; Melanogenesis; Huntington's disease; Renal cell carcinoma; Prostate cancer |
| Pik3c2b_predicted | phosphoinositide-3-kinase, class 2, beta polypeptide (predicted) | NM_001105951 /// XM_001060466 /// XM_213879 | −1.876854528 | −0.773322896 | Phosphatidylinositol signaling system |
| Vamp4_predicted | vesicle-associated membrane protein 4 (predicted) | NM_001108856 /// XM_001070325 /// XM_344168 | −1.409909017 | −1.103830892 | SNARE interactions in vesicular transport |
| Ddx52 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 52 | NM_053525 | −1.366772065 | −1.008179366 | Starch and sucrose metabolism; Folate biosynthesis |
| Sp1 | Sp1 transcription factor | NM_012655 | −1.235411929 | −0.949305052 | TGF-beta signaling pathway |

Ccnb2 and Cdc2a were suggested as cell cycle-related genes, which may be involved in cancer inhibition by I3C at an early stage.

Furthermore, I3C treatment inhibited MAPK signaling pathway-related genes, such as Fgfr1 and Pdgfrb, which were also up-regulated by DMBA treatment. Fgfr1 is a transmembrane receptor that mediates the signal transduction of fibroblast growth factor and part of the tyrosine kinase receptor, that is related to tumor formation and affects cellular regulation such as proliferation, apoptosis, cell movement, and angiogenesis[36]. Moreover, Pdgfrb binds to the platelet-derived growth factor BB homodimer, which is related to the formation of breast cancer[37]. Although Ppp5c acts as a phosphatase and inactivates atrial natriuretic peptide receptor/guanylyl cyclase[38], no relationship with tumor formation has been reported until now.

Additionally, I3C treatment inhibited insulin signaling pathway genes such as Ppp1r3b and Flot2, which were up-regulated by DMBA treatment. Ppp1r3b is a subunit of protein phosphatase-1 that is involved in hepatic glycogen-related activity[39]; however, no relationship with tumor formation has been reported. Also Flot2 is a GPI-related cell adhesion molecule and is related to the metastasis of squamous cell carcinoma in head and neck[40].

On the other hand, I3C treatment induced recovery to control level of Tgfbr1 and Ppp3cb which were down-regulated by DMBA treatment. TGF-beta binds to TGF-beta receptor and acts as an important factor in cell cycle, cell differentiation, matrix formation, growth inhibition, angiogenesis, immune suppression and apoptosis. Tgfbr1, as a TGF-beta receptor, inhibits liver tumor formation[41,42]. It has also been thought that Ppp3cb is a protein phosphatase[43], however, no clear association with tumor formation has been reported.

Taken together, we have identified several genes involving the cell cycle, MAPK signaling pathway, insulin signaling pathway, and TGF-beta signaling pathway, as implicated in the chemopreventive effect of I3C in mammary carcinogenesis at an early stage. Further studies are thus warranted to analyze the functions of these genes using siRNA in DMBA-induced carcinogenesis, such that any chemopreventive strategy will be based on a firm foundation.

## Materials & Methods

### Animals and Chemicals

Five week-old female Sprague-Dawley (SD) rats were obtained from Charles River Japan, Inc. (Atsugi, Japan), and housed in a room maintained on a 12-h light/dark cycle, at constant temperature and humidity. They were allowed free access to pellet chow (MF-1, Oriental Yeast Co., Tokyo, Japan) during the experiment. I3C and DMBA were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### Treatments

Nine SD rats were divided randomly into three groups (n=3 per group). Animals of Groups 1 and 2 were maintained on MF-1 basal diet throughout the period. Animals of Group 3 were given a diet containing 300 ppm I3C from 6 weeks of age. At 7 weeks of age, animals of Groups 2 and 3 were orally intubated with DMBA (10 mg/rat) solved in sesame oil; animals of Group 1 were injected with sesame oil only. All animals were sacrificed at 2 weeks after I3C treatment.

Animals were fed with or without 300 ppm I3C in their diet and were sacrificed at 8 weeks of age. At necropsy, the mammary glands from each group were fixed in 10% phosphate-buffered formalin and processed routinely for embedding in paraffin, and 4-μm sections were stained with hematoxylin and eosin for histopathological examination. Mammary gland samples from all animals were also snap-frozen in liquid nitrogen for RNA extraction and subsequent analysis.

### RNA Isolation, cRNA Preparation and Microarray Hybridization

Approximately 100 mg of frozen tissue was homogenized, and total RNA was isolated using ISOGEN (Nippon Gene Co. Ltd, Tokyo, Japan), isopropanol precipitated, dissolved in DEPC-treated distilled water, and stored at −80°C until use. RNA concentrations were determined by spectrophotometry (Ultraspec 3000, UV/visible spectrophotometer; Pharmacia Biotech, Tokyo, Japan). The quality of the isolated RNA was assessed by the absorbance at 260 nm, the A260/A280 ratio (1.7-2.0), and evaluation of the integrity of 28S and 18S RNA bands on 1% agarose gels.

A 12-μg aliquot of total RNA was processed for cDNA synthesis using a cDNA synthesis kit (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. Biotin-labeled antisense cRNA was synthesized by the *in vitro* transcription reaction (IVT) using an RNA transcript labeling kit (Affymetrix), purified and fragmented, and then hybridized to an oligonucleotide microarray (Affimetrix Gene Chip®, Rat Genome 230 2.0 Array) for 16 h at 45°C according to the manufacturer's instructions. The arrays were washed and stained with R-phycoerythrin conjugated streptavidin (Molecular Probes, Eugene, OR, USA) using a Fluidics station 450 (Affymetrix) and then scanned with an Affymetrix GeneChip Scanner 3000.

Raw data were converted into cell intensity measurements using the Affymetrix GeneChip Operating System and then standardized using the MARS 5.0 algorithm. Gene expression data from the microarray were input to GenPlex software ver 3.0 (ISTECH Inc., Korea). Gene expression patterns were analyzed by hierarchical clustering, using genes extracted from each group, by one-way ANOVA ($P < 0.05$). The signal log ratio values, which represent the ratios of hybridization signals between Groups 1 and 2 or Groups 2 and 3, were calculated. Altered genes were defined as those differing by over 1.2-fold with significance ($P < 0.05$) using the unpaired Welch's t-test, and common genes were extracted by Venn diagram.

The classification of associated pathways for interesting genes was performed using the KEGG pathway database. The selected genes were annotated based on NetAffx (http://www.affymetrix.com).

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## References

1. Sugimura, T. Studies on environmental chemical carcinogenesis in Japan. *Science* **233**:312-318 (1986).
2. Sugimura, T., Wakabayashi, K., Nakagama, H. & Nagao, M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci* **95**:290-299 (2004).
3. Kelloff, G. J. *et al.* Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J Nutr* **130**:467S-471S (2000).
4. Steinmetz, K. A. & Potter, J. D. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc* **96**:1027-1039 (1996).
5. Broadbent, T. A. & Broadbent, H. S. 1-1. The chemistry and pharmacology of indole-3-carbinol (indole-3-methanol) and 3-(methoxymethyl)indole. [Part I]. *Curr Med Chem* **5**:337-352 (1998).
6. Dashwood, R. H. *et al.* Quantitative inter-relationships between aflatoxin $B_1$ carcinogen dose, indole-3-carbinol anti-carcinogen dose, target organ DNA adduction and final tumor response. *Carcinogenesis* **10**: 175-181 (1989).
7. Wargovich, M. J. *et al.* Aberrant crypts as a biomarker

for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev* **5**:355-360 (1996).
8. Kojima, T., Tanaka, T. & Mori, H. Chemoprevention of spontaneous endometrial cancer in female Donryu rats by dietary indole-3-carbinol. *Cancer Res* **54**:1446-1449 (1994).
9. Kang, J. S. *et al.* Pre-initiation treatment of indole-3-carbinol (I3C) inhibits 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary carcinogenesis. *Korean J Vet Res* **41**:549-555 (2001).
10. Grubbs, C. J. *et al.* Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. *Anticancer Res* **15**:709-716 (1995).
11. Bradlow, H. L., Michnovicz, J., Telang, N. T. & Osborne, M. P. Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* **12**:1571-1574 (1991).
12. Vang, O., Jensen, M. B. & Autrup, H. Induction of cytochrome P450IA1 in rat colon and liver by indole-3-carbinol and 5,6-benzoflavone. *Carcinogenesis* **11**: 1259-1263 (1990).
13. Xu, M. *et al.* Inhibition of 2-amino-3-methylimidazo [4,5-*f*]quinoline-DNA adducts by indole-3-carbinol: dose-response studies in the rat colon. *Carcinogenesis* **18**:2149-2153 (1997).
14. Stresser, D. M., Bailey, G. S. & Williams, D. E. Indole-3-carbinol and beta-naphthoflavone induction of aflatoxin $B_1$ metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin $B_1$ in the rat. *Drug Metab Dispos* **22**:383-391 (1994).
15. Staack, R., Kingston, S., Wallig, M. A. & Jeffery, E. H. A comparison of the individual and collective effects of four glucosinolate breakdown products from brussels sprouts on induction of detoxification enzymes. *Toxicol Appl Pharmacol* **149**:17-23 (1998).
16. Jellinck, P. H. *et al.* Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem Pharmacol* **45**:1129-1136 (1993).
17. Cover, C. M. *et al.* Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling. *J Biol Chem* **273**:3838-3847 (1998).
18. Cover, C. M. *et al.* Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells. *Cancer Res* **59**:1244-1251 (1999).
19. Bonnesen, C., Eggleston, I. M. & Hayes, J. D. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res* **61**:6120-6130 (2001).
20. Meng, Q. *et al.* Suppression of breast cancer invasion and migration by indole-3-carbinol: associated with up-regulation of BRCA1 and E-cadherin/catenin complexes. *J Mol Med* **78**:155-165 (2000).
21. Kang, J. S. *et al.* Post-initiation treatment of Indole-3-carbinol did not suppress *N*-methyl-*N*-nitrosourea induced mammary carcinogenesis in rats. *Cancer Lett* **169**:147-154 (2001).
22. Malejka-Giganti, D., Niehans, G. A., Reichert, M. A. & Bliss, R. L. Post-initiation treatment of rats with indole-3-carbinol or beta-naphthoflavone does not suppress 7, 12-dimethylbenz[*a*]anthracene-induced mammary gland carcinogenesis. *Cancer Lett* **160**:209-218 (2000).
23. Kang, J. S., Kim, D. J. & Jang, D. D. The modifying effect of indole-3-carbinol (I3C) and its metabolites, phytochemicals from cruciferae vegetables, in chemical carcinogenesis and cancer prevention. *J of Korean Association of Cancer Prevention* **5**:199-208 (2000).
24. Dashwood, R. H. Indole-3-carbinol: anticarcinogen or tumor promoter in brassica vegetables? *Chem Biol Interact* **110**:1-5 (1998).
25. Bjeldanes, L. F. *et al.* Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci USA* **88**:9543-9547 (1991).
26. Butte, A. The use and analysis of microarray data. *Nat Rev Drug Discov* **1**:951-960 (2002).
27. Clarke, P. A., te Poele, R. & Workman, P. Gene expression microarray technologies in the development of new therapeutic agents. *Eur J Cancer* **40**:2560-2591 (2004).
28. Ellinger-Ziegelbauer, H. *et al.* Characteristic expression profiles induced by genotoxic carcinogens in rat liver. *Toxicol Sci* **77**:19-34 (2004).
29. Bulera, S. J. *et al.* RNA expression in the early characterization of hepatotoxicants in Wistar rats by high-density DNA microarrays. *Hepatology* **33**:1239-1258 (2001).
30. Michel, C. *et al.* Liver gene expression profiles of rats treated with clofibric acid: comparison of whole liver and laser capture microdissected liver. *Am J Pathol* **163**:2191-2199 (2003).
31. Tilton, S. C. *et al.* Toxicogenomic Profiling of the Hepatic Tumor Promoters Indole-3-Carbinol, 17beta-Estradiol and beta-Naphthoflavone in Rainbow Trout. *Toxicol Sci* **90**:61-72 (2006).
32. Lippert, T. H., Seeger, H. & Mueck, A. O. The impact of endogenous estradiol metabolites on carcinogenesis. *Steroids* **65**:357-369 (2000).
33. Kang, J. S. *et al.* Inhibition of mammary gland tumors by short-term treatment of estradiol-3-benzoate associated with down-regulation of estrogen receptor ERalpha and ERbeta. *Oncol Rep* **12**:689-693 (2004).
34. Hilakivi-Clarke, L. *et al.* Do estrogens always increase breast cancer risk? *J Steroid Biochem Mol Biol* **80**:163-174 (2002).
35. He, Y. H., Smale, M. H. & Schut, H. A. Chemopreventive properties of indole-3-carbinol (I3C): inhibition of DNA adduct formation of the dietary carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), in female F344 rats. *J Cell Biochem Suppl* **27**:42-51 (1997).

36. Acevedo, V. D., Ittmann, M. & Spencer, D. M. Paths of FGFR-driven tumorigenesis. *Cell Cycle* **8**:580-588 (2009).
37. de Jong, J. S., van Diest, P. J., van der Valk, P. & Baak, J. P. Expression of growth factors, growth factor receptors and apoptosis related proteins in invasive breast cancer: relation to apoptotic rate. *Breast Cancer Res Treat* **66**:201-208 (2001).
38. Chinkers, M. Targeting of a distinctive protein-serine phosphatase to the protein kinase-like domain of the atrial natriuretic peptide receptor. *Proc Natl Acad Sci USA* **91**:11075-11079 (1994).
39. Munro, S. *et al.* A novel glycogen-targeting subunit of protein phosphatase 1 that is regulated by insulin and shows differential tissue distribution in humans and rodents. *Febs J* **272**:1478-1489 (2005).
40. Rickman, D. S. *et al.* Prediction of future metastasis and molecular characterization of head and neck squamous-cell carcinoma based on transcriptome and genome analysis by microarrays. *Oncogene* **27**:6607-6622 (2008).
41. Reisenbichler, H., Chari, R. S., Boyer, I. J. & Jirtle, R. L. Transforming growth factor-beta receptors type I, II and III in phenobarbital-promoted rat liver tumors. *Carcinogenesis* **15**:2763-2767 (1994).
42. Mansbach, J. M. *et al.* Phenobarbital selectively promotes initiated cells with reduced TGF beta receptor levels. *Carcinogenesis* **17**:171-174 (1996).
43. Wadzinski, B. E., Heasley, L. E. & Johnson, G. L. Multiplicity of protein serine-threonine phosphatases in PC12 pheochromocytoma and FTO-2B hepatoma cells. *J Biol Chem* **265**:21504-21508 (1990).