Gene signature of rat mammary glands: Influence of lifelong soy isoflavones consumption

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Abstract. Epidemiological studies have indicated that phytoestrogen has a preventive effect on breast cancer development. However, controversial results have been reported suggesting these compounds have ambivalent effects on breast tissue. Here, we report a transgenerational study conducted on female Wistar rats fed a diet enriched with phytoestrogen. Using a pangenomic microarray approach, a transcriptomic study was performed on mammary glands extracted from the animals. Gene expression was examined at 3 ages: 3, 18 and 24 months. The F_1 generation did not express the same genes as the F_0 control generation fed the same diet. This effect increased with animal age: in 3-, 18- and 24-month-old rats, 293, 441 and 2868 differentially expressed genes were respectively observed. These results suggest that long-term exposure to isoflavones may play a key role in gene regulation. Additionally, epigenetic patterns were found to be affected by DNA-methyltransferase and histone-deacetylase expression.

Introduction

Breast cancer is the most common cause of female cancer mortality in the world, with 115,000 cases diagnosed in 2002 (1). The identification of preventive actions is a key challenge in breast cancer research. One potential strategy for the prevention of breast cancer is the application of diets adapted specifically to individuals. Indeed, lifestyle changes, particularly diet during early life, may significantly affect cancer risk during later years (2). Our research focused on the bioactive nutrients genistein and daidzein, the two main soy isoflavones.

Epidemiological studies have demonstrated that breast cancer incidence is higher in western countries than in Asian countries such as China or Japan. This can be attributed to environmental factors and to the high intake of soy, which is rich in genistein and daidzein, in Asian countries (3-5). Genistein and daidzein belong to the isoflavone class of flavanoids and may be classified as phytoestrogens due to their structural similarities to estradiol. In fact, at high concentrations *in vitro*, genistein and daidzein have been shown to exert estrogenic effects greater than estradiol (6-8).

The effect of genistein on the mammary glands was also explored in *in vivo* experiments, in which 17β-estradiol and genistein were observed to share many effects in the mammary glands, increasing proliferation and progesterone receptor expression (9). Moreover, Kim *et al* demonstrated that exposure to genistein in the prepubertal period inhibits mammary epithelial cell proliferation (10). However, other studies have reported ambivalent effects of phytoestrogens on breast tissue. Hertrampf *et al* performed a study on genistein using non-tumour bearing ovariectomised female Wistar rats (11). In non-malignant breast tissue, short-term administration of genistein did not induce proliferation. The authors concluded that the chronic stimulation of proliferation is potentially a key mechanism behind breast cancer development. Studies on genistein that performed gene profiling in the rat mammary glands showed that genistein treatment induced changes in gene expression (12,13). In order to elucidate the action of phytoestrogens on gene expression over generations, we performed an *in vivo* transcriptomic study on two generations of female Wistar rats fed a diet enriched with isoflavones.

Materials and methods

Animals and diets. The study was conducted in accordance with current legislation on animal experiments in France. A total of 60 female Wistar rats (F_0 generation, 3 months old) were purchased from the animal husbandry department of

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Table I. Composition of the soy protein-free powdered semi-purified diet.

Ingredients[a]	g/kg
Casein	200
Sucrose	220
Cornstarch	440
Cellulose fiber	50
Peanut oil	25
Rapeseed oil	25
Vitamin mixture[b]	10
Mineral mixture[c]	25
DL-Methionine	3
Choline bitartrate	2

[a]Casein (Union des caseineries, Surgeres, France), sucrose (Eurosucre, Paris, France), cornstarch (Cerestar, Saint-Maur, France), cellulose (Durieux, Marne la Vallée, France), oil (Bailly, Aulnay sous Bois, France), vitamin mixture (Roche, Neuilly sur Seine, France), mineral mixture (Prolabo, Fontenay sous Bois, France) and DL-methionine (Jerafrance, Jeufosse, France). [b]Expressed in mg/kg of mixture: retinyl palmitate (250 IU/mg), 2,000; cholecalciferol (400 UI/mg), 312; DL-α-tocopherol acetate (0.25I U/mg), 20,000; menadione, 100; thiamine HCL, 1,000; riboflavin, 1,000; nicotinic acid, 4,500; D-calcium pantothenate, 3,000; pyridoxine HCL, 1,000; inositol, 5,000; D-biotin, 20; folic acid, 200; cyanocobalamin, 1.35; ascorbic acid, 10,000; p-aminobenzoic acid, 5,000; choline chlorhyascorbic acid, 10,000; p-aminobenzoic acid, 5,000; choline chlorhydrate, 75,000; and sucrose, finely powdered, 871.9 g. [c]Expressed in g/kg of mixture: $CaHPO_4 \bullet {}_2H_2O$, 308; K_2Hpo_4, 194; $CaCO_3$, 146; $MgSO_4 \bullet 7H_2O$, 109; NaCl, 168; MgO, 24.3; $FeSO_4 \bullet 7H_2O$, 20.9; $ZnSO_4 \bullet H_2O$, 12.1; $MnSO_4 \bullet H_2O$, 12.1; $CuSO_4 \bullet 5H_2O$, 0.0005; CoCO3, 0.0005; Na_2SeO_3, 0.0005.

the Institut National de la Recherche Agronomique (INRA; Clermont-Ferrand/Theix, France). Animals were housed in plastic cages (8 rats/cage) at 25˚C with a relative humidity of 55% under a 12:12 h light-dark cycle. These F_0 generation rats were randomly divided in two groups (30 rats/group) fed *ad libitum* with a standard diet (dry food from INRA, Jouy en Josas, France). The food of the first (control) group did not contain any soy proteins (these were replaced by casein; Table I), while the second (isoflavones) group received food enriched with isoflavones (ADM: Novasoy Isoflavones compound 152-400; Archer Dianels Midland Company, Decatur, IL, USA). The total isoflavones (genistein 159, daidzein 156, glycitin 33) were present at a concentration of 348 mg/g; i.e., 0.87 mg of isoflavones/g of diet. The animals had free access to water during the entire experimental period, and their body weight and food intake were measured once a week. After a 30-day adaptation period, the females were allowed to mate, and were maintained on the same regimen for the entire gestation and lactation period. At weaning, female offspring (the F_1 generation) born into each experimental group (control and isofalvones) were further divided into two groups, for a total of four nutritional groups (Fig. 1) fed either the standard (control) diet or the isoflavones rich diet. At 3, 18 and 24 months after birth, and 2 months after parturition for the F_1 generation, 10 animals in each group were intraperitonally anaesthetized with chloral hydrate. Mammary glands were removed, snap frozen and maintained at -140˚C until analysis (14).

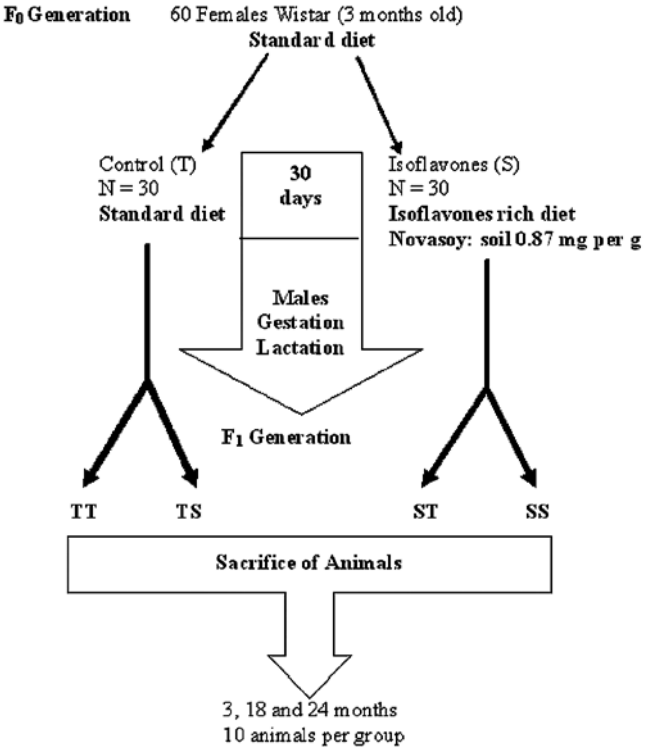

Figure 1. Experimental protocol.

Total RNA extraction. The mammary gland of each rat was weighed quickly and ground thoroughly with a French press. Power was disrupted in a potter with the appropriate quantity of RTL buffer with the addition of β-mercaptoethanol, according to the weight of the gland. The solution was homogenized by passing lysate at least 10 times through a 30-needle fitted to an RNase-free syringe. Total mRNA isolation was performed with the RNA easy mini kit (Qiagen) following the manufacterer's protocol. Integrity of the RNA samples was verified using a 2100 BioAnalyzer with RNA 6000 Nano LabChip® and BioSizing A.02.11 software (Agilent Technologies).

cRNA amplification, labeling and hybridization. Total RNA (1 μg) was amplified and labeled using the Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies) according to the manufacturer's intructions. Each amplified cRNA was fluorescently labeled with Cy3-CTP (Perkin Elmer®). A rat RNA reference (Stratagene®) was used to normalize gene expression, and was then Cy5-labeled. Both Cy3 and Cy5 probes were hybridized on whole rat genome arrays (Agilent Technologies). Hybridization was carried out in an oven at 60˚C overnight. After a first wash in GE Wash Buffer 1 at room temperature for 1 min and a second wash in GE Wash Buffer 2 (both from Agilent Technologies) at an elevated temperature for 1 min, slides were incubated in a stabilization and drying solution (Agilent

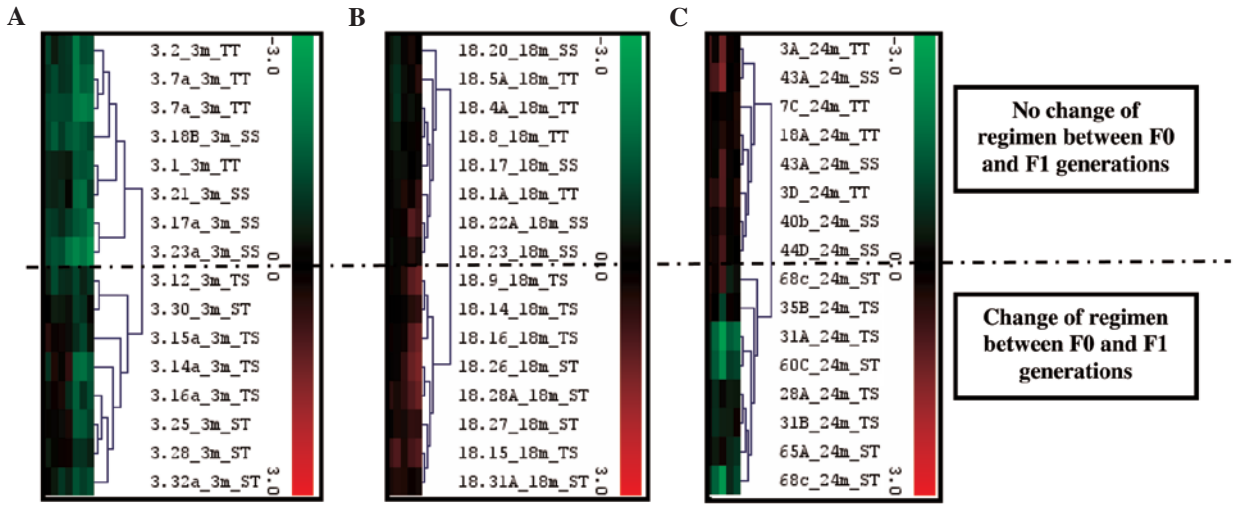

Figure 2. Clusters of (A) 3-month-, (B) 18-month- and (C) 24-month-old groups according to the four regimens (SS: soy-soy; TT: control-control; TS: control-soy; ST: soy-control). Hierarchical analysis clustered all groups with or without change of regimen between the two generations.

Technologies) to remove excess buffer and to stabilize the cyanine incorporation. Each array (whole rat genome 44K; Agilent Technologies) was performed in triplicate: each RNA sample was hybridized three times in order to produce three technical replicates. Four RNA samples per nutritional regimen were hybridized, for a total of 16 microarrays. Fluorescence intensity was determined with a 418 GMS scanner (Affymetrix®) and quantitative values for the signals were calculated using GenePix Pro 6 (Axon®) software. Expression ratios between rat mammary gland and the reference were obtained for each spot in a GenePix file.

Analysis of gene expression. Analysis was performed using limmaGUI Version 1.7.0 software, Limma Version 2.4.4 package and the R 2.2.0 program (15). After being $\log_2$-transformed, the ratio of Cy3 to Cy5 signal intensities (Cy3/Cy5) was normalized by the global LOWESS method based on the local estimation of intensities and a regression calculation weighted towards similar spots. Gene probes used as negative controls were removed from the data set, and hierarchical clustering was performed with TIGR MeV version 3.0.3 software (The Institute for Genomic Research©) using a Pearson correlation and the complete linkage clustering method. The cluster was color-coded red for up-regulation and green for down-regulation as compared to the control group. Significant genes were clustered by the t-test.

We preferentially explored epigenetic phenomena via DNA methyltransferase (DNMT) and histone acetylase (HDAC) variant expression. DNA methylation has been studied through two sub-types of methyltransferases, those involved in a pre-existing pattern of methylation during replication (DNMT1) and those mainly involved in the *de novo* methylation of CpG islands (DNMT3a, -3b and -3l), largely associated with tumorigenesis. For HDAC expression, we focused on two classes of HDAC: class I consisting of HDAC1, -2 and -3, and class II consisting of HDAC4, -5, -6 and -7a. HDAC2 is also expressed in breast tissue and largely interacts with DNMT1. HDAC3 and -4 are implicated in cell-cycle progression, DNA damage and apoptosis.

Results

Differentially expressed genes according to age. To assess the genes involved in each group, we performed a t-test analysis in 3-, 18- and 24-month-old rats that underwent regimen change or not between the two generations (Figure 2A, B and C). Fig. 2 shows the hierarchical clustering of all samples. This analysis permitted us to distinguish groups affected or not by change of regimen between the two generations. Changes in diet were observed to have an effect between generations on mammary gland gene expression. F_0 and F_1 generation rats fed the same regimen (TT and SS) were classified in one group compared to rats undergoing a change of regimen between the two generations (TS and ST). As a second method, we performed an analysis of the effects of age on gene expression. The number of expressed genes was found to increase according to age group: 293 genes involved in 38 molecular pathways for the 3-month-old rats, 441 genes involved in 84 molecular pathways for the 18-month-old rats, and 2,868 genes involved in 135 molecular pathways for the 24-month-old rats. These genes were classified by molecular pathway. Twenty-four common pathways were observed to be induced among the three age groups (Table II). The selected genes had a p-value $<10^{-3}$.

DNMT and HDAC expression. To elucidate whether transgenerational diet changes are capable of determining the epigenetic reprogramming of the embryonic transcriptome, the expression of DNA methyltransferase (*DNMT1*, *-3a*, *-3b* and *-3l*) and histone acetylase (*HDAC2*, *-3*, *-4*, *-5*, *-6* and *-7a*) was examined. A decrease in *DNMT* was observed in the three age groups (Fig. 3A, B and C), with the exception of rats fed the TS regimen. In these rats, *DNMT1* and *-3a* expression was increased in rats aged 18- and 24-months (*DNMT3a* only). With regard to *HDAC* (Fig. 4A, B and C), a decrease in *HDAC2* was found in the three age groups, with the exception of 24-month-old rats fed the TS regimen. *HDAC3*, *-4* and *-5* expression was increased in all groups. A decrease in *HDAC6* expression was noted in 3- and 24-month-old rats, while

Table II. Common pathways induced and differentially expressed genes.

Function	Genes
Adherens junction	*ACTN3; EGFR, ERBB; LMO7, FBXO20; LIM domain 7; TGFBR; WAS, IMD2, THC*;
Alkaloid biosynthesis II	*AADAC; AOC3; PRDX6*;
β-alanine metabolism	*ACADM; ALDH3A1, ALDH3; AOC3; DPYD*;
Calcium signaling pathway	*ADCY3; ADRA1A, ADRA1C; CACNA1B, CACNL1A5; CACNA1F, CSNB2; CALM2; EGFR, ERBB; ERBB4;HRH1; ITPR2; LHCGR; P2RX2; P2RX3; PDE1A; PDE1C; PLCE1; PLN; RYR2, ARVD2; SLC25A4,PEO3, PEO2, ANT1; SLC25A5, ANT2*;
Chronic myeloid leukemia	*AKT3; IKBKG, IP2, IP1; MAP2K1; PIK3CA; PIK3R2; RB1; RELA; STAT5A, STAT5; TGFB1, TGFB, DPD1; TGFBR1*;
Colorectal cancer	*AKT3; AXIN2; CASP3; EGFR, ERBB; FZD2; MAP2K1; PIK3CA; PIK3R2; TGFB1, TGFB, DPD1; TGFBR1*;
Cytokine-cytokine receptor interaction	*BMPR1A, ACVRLK3; EGFR, ERBB; FLT4; GHR; IL13RA1; IL23A; IL2RA, IL2R; IL6R; IL9R; KIT; LEPR; TGFB1, TGFB, DPD1; TGFBR1; TNFRSF8; VEGFA, VEGF*;
Focal adhesion	*ACTN3; AKT3; BCAR1; EGFR, ERBB; ELK1; IGF1; ITGA4; MAP2K1; MYL2; PIK3CA; PIK3R2; VEGFA,VEGF*;
Hedgehog signaling pathway	*CSNK1A1; SMO, SMOH; WNT5B*;
Gap junction	*ADCY3; ADCY5; EGFR, ERBB; GUCY1A2; ITPR2; MAP2K1*;
Glioma	*AKT3; CALM2; EGFR, ERBB; IGF1; MAP2K1; PIK3CA; PIK3R2; RB1*;
Leukocyte transendothelial migration	*VIL2*;
Long-term depression	*GUCY1A2; IGF1; ITPR2; MAP2K1; PPP2CB*;
MAPK signaling pathway	*AKT3; CACNA1B, CACNL1A5; CACNA1F, CSNB2; CACNA2D2; CACNB2, MYSB, CACNLB2; CACNG5; CASP3; DUSP7; EGFR, ERBB; ELK1; FGF20; FGF23; FGFR3, ACH; GNG12; IKBKG, IP2, IP1; MAP2K1; MAPK14; RASGRF2; STMN1, LAP18; TGFB1, TGFB, DPD1; TGFBR1*;
Melanoma	*AKT3; EGFR, ERBB; FGF20; FGF23; IGF1; MAP2K1; PIK3CA; PIK3R2; RB1*;
mTOR signaling pathway	*AKT3; IGF1; PIK3CA; PIK3R2; PRKAA2, PRKAA; RPS6KA1; TSC1; ULK1; VEGFA, VEGF*;
Pancreatic cancer	*AKT3; BRCA2, FANCD1, FACD, FANCD; EGFR, ERBB; IKBKG, IP2, IP1; MAP2K1; PIK3CA; PIK3R2; RB1; RELA; TGFB1,TGFB, DPD1; TGFBR1; VEGFA, VEGF*;
Prostate cancer	*AKT3; CREB1; EGFR, ERBB; IGF1; IKBKG, IP2, IP1; MAP2K1; PIK3CA; PIK3R2; RB1; RELA*;
Purine metabolism	*ADCY3; ADCY5; GUCY1A2; NUDT9; PDE1A; PDE11A; PDE1C; PDE4A; PDE4D; PDE9A*;
Regulation of actin cytoskeleton	*VIL2*;
Ribosome	*RPL29; RPL37; RPS10; RPS27; RPS5; RPS9*;
Tight junction	*ACTN3; AKT3; CASK; CLDN16; CSDA; LLGL1, DLG4, LLGL, HUGL, HUGL-1; MYH1; MYH10; MYH4; MYL2; PPP2CB; PPP2R2C; PRKCQ; VAPA; VAMP*;
Tryptophan metabolism	*AADAT; ACAT1, ACAT; ALDH3A1, ALDH3; AOC3; ASMT; CYP1A2; HAAO; INDO, IDO; KYNU*;
Wnt signaling pathway	*AXIN2; CSNK1A1; FZD2; PPP2CB; PPP2R2C; RUVBL1; SENP2; SUMO1, SMT3; SFRP1; WNT5B*;

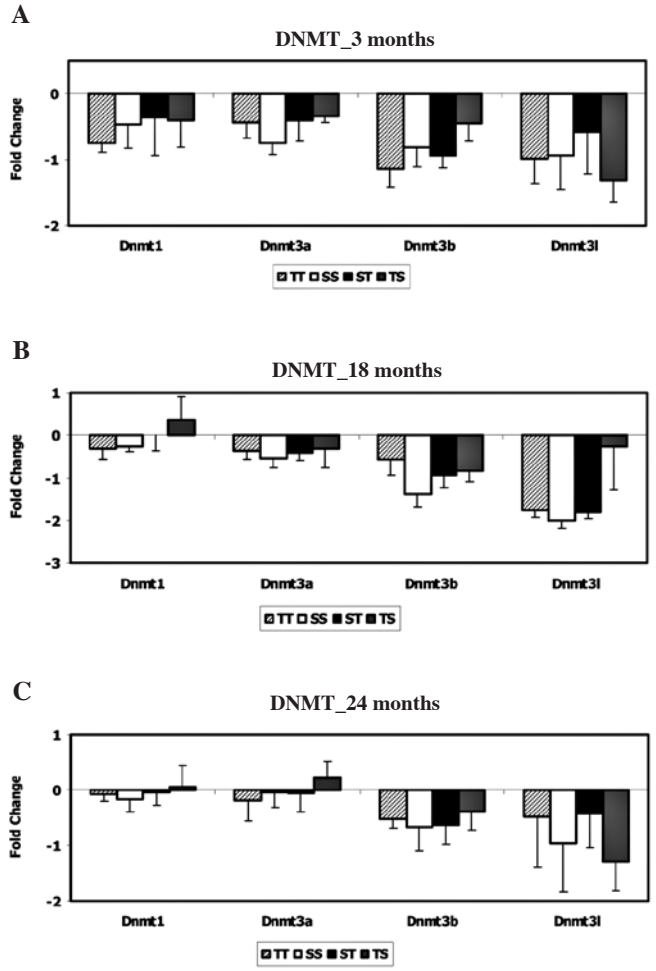

Figure 3. Fold changes of DNMT observed for (A) 3-month-, (B) 18-month- and (C) 24-month-old rats according to the TT, SS, ST or TS regimen.

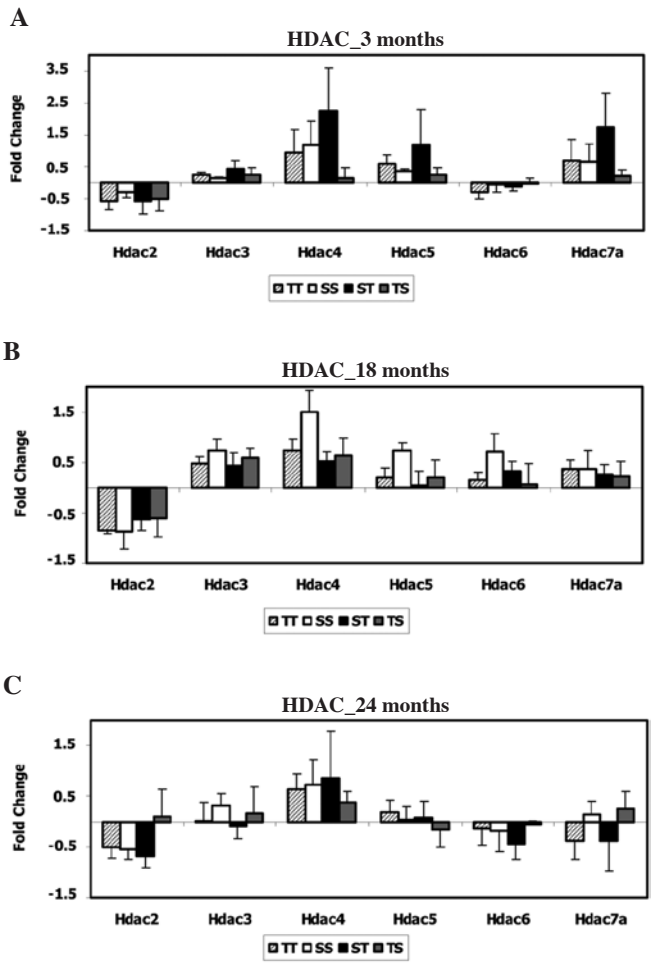

Figure 4. Fold changes of HDAC observed for (A) 3-month-, (B) 18-month- and (C) 24-month-old rats according to the TT, SS, ST or TS regimen.

an increase was observed in 18-month-old rats. *HDAC7a* expression was increased in all the 3- and 18-month-old rats, while in the 24-month-old rats it was only increased in those receiving the SS and TS regimens.

Discussion

We performed a transgenerational *in vivo* study using a pangenomic approach on female Wistar rats fed a phytoestrogen-enriched diet. Two groups that underwent a regimen change between two generations were observed. Rats fed identically between the F_0 and F_1 generations (TT and SS) were classified in the same group compared to rats fed a different regimen between the two generations (TS and ST). The number of differentially expressed genes was observed to increase based on the age of the rats. These results suggest that long-term exposure to isoflavones may play a key role in gene regulation. Among the three age groups, several genes were found to be differentially expressed in common, including epithelial growth factor (*EGFR*), v-AKT murine thymoma viral oncogene homolog 3 (*AKT3*), phosphoinositide 3-kinases (*PIK3CA*), insuline growth-factor 1 (*IGF-1*), V-ERB-B2 avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*), retinoblastoma (*RB*), mitogen-activated protein kinase (*MAPK*), transforming growth factor β1 (*TGFβ1*) and vascular endothelial growth factor (*VEGF*). EGFR expression was examined following genistein treatment, and genistein, an inhibitor of tyrosine kinase activity, was shown to inhibit EGF-induced tyrosine phosphorylation and the degradation of EGFR in HepG2 cells. This suggests that tyrosine kinase activity is required for the internalization or degradation of EGF-EGFR receptor complexes (16). Sordella *et al* (17) reported that mutant EGFRs selectively activate the AKT and STAT signaling pathways, which promote cell survival. However, mutant EGFRs had no effect on extracellular signal-regulated kinase signaling, which induces proliferation. In a previous study, we also observed the phytoestrogen-modulated expression of *AKT3* to be implicated in numerous biological processes, including adipocyte and muscle differentiation, glycogen synthesis, glucose uptake, apoptosis and cellular proliferation (18).

Our results indicate a modification of *IGF1* expression. *In vitro*, low levels of IGF1 prevent VEGF-induced activation of protein kinase B (AKT1), a kinase critical for endothelial cell survival. Hankinson *et al* (19) demonstrated a strong association between circulating IGF1 concentrations and the risk of premenopausal breast cancer. This is not the case in postmenopausal women. In an accompanying commentary, Holly (20) discussed the evidence regarding whether high levels of circulating IGF1 are capable of increasing the risk

of breast cancer in premenopausal women, and noted that a similar association had been reported for prostate cancer. Arends *et al* (21) hypothesized that minor genetic variations in the IGF1 gene are capable of influencing prenatal and postnatal growth. Since low IGF1 levels are associated with type 2 diabetes and cardiovascular disease, the authors proposed that the IGF1 gene may provide a link between low birth weight and the occurrence of these diseases in later life.

We also observed a modification in breast cancer-related genes such as *ERBB2* and *RB*. Hanahan and Weinberg (22) reported that the *RB* retinoblastoma gene, which was the first tumor suppressor gene cloned, is a negative regulator of the cell cycle due to its ability to bind the transcription factor E2F and to repress the transcription of genes required for the S phase. These authors referred to the deregulation of the retinoblastoma protein pathway as a 'hallmark of cancer'. In the absence of other genetic alterations, deregulation results in a lack of differentiation, hyperproliferation and apoptosis. The RB protein acts as a transcriptional repressor by targeting the E2F transcription factors, the functions of which are required for entry into the S phase. *ERBB2* is an oncogene responsible for 1/3 of all female cancers and approximatively 1/4 of cancer-related deaths in women (23). Our results confirm the protective effect of isoflavones against breat cancer during fetal life, and suggest they have a role in breast carcinogenesis.

Phytoestrogen supplementation may also affect cell cycle regulation, as we observed a modification of *TGFβ* and *VEGF* expression. *TGFβ* is a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. Dysregulation of TGFβ activation and signaling may result in apoptosis. Derynck *et al* (24) reviewed TGFβ signaling in tumor suppression and cancer progression. VEGF may be a major regulator of tumor angiogenesis *in vivo*, and mediates angiogenic activity in a variety of estrogen target tissues. To determine whether estrogen has a direct transcriptional effect on VEGF gene expression, Mueller *et al* (25) demonstrated that estradiol (E2)-regulated *VEGF* gene transcription requires a variant estrogen response element (ERE) located 1.5 kb upstream from the transcriptional start site. Site-directed mutagenesis of this ERE abrogated E2-induced *VEGF* gene expression. *VEGF* and TGFβ1 have opposing effects on endothelial cells, in that *TGFβ1* induces apoptosis and *VEGF* protects endothelial cells from apoptosis. However, they are often co-expressed in angiogenic tissues, and *TGFβ1* up-regulates *VEGF* expression. Using bovine and human endothelial cells, Ferrari *et al* (26) found that crosstalk between *TGFβ1* and *VEGF* is capable of converting *VEGF* into a proapoptotic signal through *VEGFR2* and *MAPK14*. In the present study, we also observed a modification of *MAPK* expression, which acted as an integration point for multiple biochemical signals. This corroborates the theory that phytoestrogen acts preventively on a variety of biological pathways.

In epigenetics, methylation patterns are established during early embryogenesis and gametogenesis. DNMT1 is responsible for cytosine methylation in mammals and has a role in gene silencing. DNMT1 is principally involved in maintaining the pre-existing methylation pattern during replication, whereas DNMT3a and -3b are mainly involved in the *de novo* methylation of CpG islands. Aberrant *de novo* methylation of growth regulatory genes is associated with tumorigenesis in humans (27). In a variety of cancers, it has been established that DNMT (mainly *DNMT1* and *-3b*) overexpression induces CpG hypermethylation and cell transformation. In our experiments, we oberved a general decrease in *DNMT* expression, which may be explained by the soy-enriched diet. It has previously been demonstrated that genistein (20-50 μmol/l) dose-dependently inhibits DNMT activity (28). The precise molecular mechanisms of genistein inhibitory interaction with human DNMT is as yet unknown, and requires further investigation. Nevertheless, this effect has also been observed in *in vivo* and *in vitro* studies. It has been demonstrated that genistein is capable of affecting DNA methylation patterns in anonymous novel CpG islands in mouse prostate DNA (29). Moreover, in a transgenerational study, epigenetic modifications were observed to have an effect on coat color in Agouti mice *in vivo* (30). We also investigated the effects of genistein and daidzein on *HDAC* expression.

As with DNA methylation, disruption of histone acetylation patterns is a common feature of cancer cells. Histone acetylation and deacetylation alternately expose and occlude DNA to transcription factors. In our study, HDAC expression was differentially modulated according to the specific groups. Indeed, we observed that for 3- and 18-month-old rats, there was an increase in *HDAC* expression, with the exception of *HDAC2* and *-6*. In 24-month-old rats, an increase in *HDAC3*, *-4* and *-5* expression and a decrease in *HDAC2*, *-6* and *-7* expression was observed. Rountree *et al* (31) demonstrated that HDAC2 interferes with DNMT1, establishing a repressive transcription complex. Previously, we showed that *DNMT1* expression was decreased, which could explain the decrease in *HDAC2* expression. Moreover, HDAC2 only joins DNMT1 during the late S phase, providing a platform for the deacetylation of histones in heterochromatin following replication. HDAC2 is more important in breast cancer, since the presence of the HDAC2 frameshift mutation is capable of causing a loss of HDAC2 protein expression and enzymatic activity. This could result in the induction of resistance to the antiproliferative and pro-apoptotic effects of histone deacetylase inhibitors, such as phytoestrogens. We observed this decrease in the three age groups, which suggests that HDAC2, -6 and -7 participate in the activation of transcription factors.

In conclusion, the present study provides some answers regarding the transgenerational effects of diet on the mammary gland. We demonstrated that phytoestrogen supplementation during fetal life is capable of modifying gene expression, particularly if a change in dietary habits occurs between two generations and the epigenetic pattern of development. Nevertheless, further investigations are needed, especially in breast cancer models, to determine potential breast cancer biomarkers.

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