Identification of biomarkers regulated by rexinoids (LGD1069, LG100268 and Ro25-7386) in human breast cells using Affymetrix microarray

HYE-SOOK SEO¹, JONG-KYU WOO², YONG CHEOL SHIN¹ and SEONG-GYU KO¹

¹Laboratory of Clinical Biology and Pharmacogenomics and Center for Clinical Research and Genomics, College of Korean Medicine, Kyung Hee University, Dongdaemun-gu, Seoul 130-701; ²Laboratory of Preventive Pharmacy, College of Pharmacy, Gachon University of Medicine and Science, Yeonsu-gu, Incheon 406-840, Republic of Korea

Received June 11, 2014; Accepted February 26, 2015

DOI: 10.3892/mmr.2015.3480

Abstract. Retinoids possess anti-proliferative properties, which suggests that they possess chemopreventive and therapeutic potential against cancer. In the current study, genes modulated by rexinoids (retinoid X receptor (RXR)-pan agonists, LGD1069 and LG100268; and the RXRα agonist, Ro25-7386) were identified using an Affymetrix microarray in normal and malignant breast cells. It was observed that LGD1069, LG100268 and Ro25-7386 suppressed the growth of breast cells. Secondly, several rexinoid-regulated genes were identified, which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus. These genes may be associated with the growth-suppressive activity of rexinoids. Therefore, the identified genes may serve as biomarkers and novel molecular targets for the prevention and treatment of breast cancer.

Introduction

Breast cancer is the most commonly diagnosed type of cancer in females and is the leading cause of cancer-related mortality in females worldwide (1). In 2013, the American Cancer Society estimated that 232,340 females would be newly diagnosed with breast cancer and 39,620 females would succumb to the disease (2). The key objectives of scientists and clinicians in managing this breast cancer are to prevent the incidence, detect it early and treat it with effective therapeutic strategies resulting in long overall survival with minimal side effects. Therefore, the aim of the current study was to identify the genes associated with cell growth inhibition that are induced by Retinoid X receptor (RXR)-selective retinoids (rexinoids), with an aim to improve prevention and treatment of breast cancer.

Retinoids regulate a variety of biological functions, including embryogenesis, growth, differentiation, vision and reproduction (3-5). Retinoids additionally possess antiproliferative properties, which suggests a chemopreventive and therapeutic role against cancer (6). In addition, retinoids have been reported to inhibit normal- or tumor-cell growth through the regulation of differentiation and/or apoptosis (7-10).

Retinoids exert their effects in target cells via interaction with retinoic acid receptors (RARs) and RXRs. Each of these includes three subtypes, termed α, β and γ, which are encoded by distinct genes. The RARα, RARβ and RARγ genes have been localized to chromosomes 17q21, 3p24 and 12q13, respectively. The RXRα, RXRβ and RXRγ genes have been mapped to chromosomes 9q34.3, 6p21.3 and 1q22-23, respectively (11). The RARs bind all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid (RA) while RXRs bind 9-cis-RA alone. RXRs are known to heterodimerize with several steroid hormone receptors, including RAR, thyroid hormone receptor, vitamin D receptor, peroxisome proliferator-activated receptor, liver X receptor, pregnane X receptor and farnesoid X receptor suggesting its involvement in several signaling pathways (12). RXRs are also able to homodimerize in transfected cells (13).

In addition to naturally occurring retinoids, including ATRA, 9-cis-RA and 13-cis-RA, various synthetic retinoids with varied selectivity have been developed and are currently available to treat psoriasis, acne, photaging, actinic keratosis and certain types of cancer, including acute promelocytic leukemia, cutaneous T-cell lymphoma and squamous or basal cell carcinoma (14). However, the use of RAR-selective retinoids is limited by their toxicity, which can result in chelitis, hypertriglyceridemia and hepatosplenomegaly (15).

Rexinoids are important in controlling apoptosis and can function in a ligand-dependent or ligand-independent manner (16,17). Notably, rexinoids have been reported to suppress estrogen receptor (ER)-positive and ER-negative...
mammary tumor development with reduced toxicity compared with RAR-selective retinoids (18-20). Retinoids are additionally active in animals with tamoxifen-resistant breast cancer (17,21) and in ATRA-resistant breast cancer cells (22). Thus, retinoids appear to be promising chemopreventive and therapeutic agents with improved efficiency as compared with RAR-selective ligands. Among the retinoids, LGD1069 (Bexarotene) was confirmed as a safe and well-tolerated agent in clinical trials of cutaneous T-cell lymphoma, breast cancer and lung cancer (22,23).

Thus, we focussed on retinoids and their cognate receptor, RXR, in breast cells, and aimed to investigate their regulatory activity on the transcription of genes involved in growth suppression. In particular, the present study investigated the RXRa isoform, which has been suggested as a potential therapeutically targeted breast cancer target, due to the observation that overexpression of RXRa sensitized breast cancer cells lines to the antiproliferative effects of RXR-selective ligands (24).

In addition, infection with adenoviral RXRa induced nucleoplasmic overexpression of RXRα and resulted in apoptosis with treatment against RXRα ligand in retinoid-resistant MDA-MB-231 cells (25). Thus, in the current study, the growth-suppressive activity of RXRα pan agonists (LGD1069 and LG100268) and an RXRα-specific ligand (Ro25-7386) were investigated in normal human mammary epithelial cells (HMECs) and four breast cancer cell lines (MCF-7, T47D, MDA-MB-231 and MDA-MB-435) using an MTS assay. Subsequently, the genes regulated by retinoids that may be involved in their antiproliferative activity were investigated with an Affymetrix microarray.

Materials and methods

Ligands and antibodies. LGD1069 and LG100268 were provided by Ligand Pharmaceuticals, Inc. (La Jolla, CA, USA). Ro25-7386 was obtained from Roche Bioscience (Palo Alto, CA, USA). These compounds were diluted in dimethyl sulfoxide purchased from Sigma-Aldrich (St. Louis, MO, USA) to a final concentration of 0.1%. Monoclonal or polyclonal antibodies (mouse or rabbit) against RXRa (cat. no. sc-553) B-cell lymphoma 2-associated X protein (Bax; cat. no. sc-7480), E-cadherin (cat. no. sc-7870), integrin α6 (cat. no. sc-13542), cell division control protein 42 (CDC42; cat. no. sc-8401) and actin (cat. no. sc-8432) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Cells and culture materials. Human normal mammary epithelial cells (HMECs) were obtained from Lonza Group (San Diego, CA, USA). Cells between passages 10 and 11 were used for experiments and the cells were grown and maintained in mammary epithelial basal medium supplemented with 13 mg/ml bovine pituitary extract, 0.5% serum, 5 µg/ml insulin, 10 ng/ml human recombinant epidermal growth factor, 0.5 mg/ml hydrocortisone, 50 µg/ml gentamicin and 50 µg/ml amphotericin-β (all Clonetics, Lonza Group, San Diego, CA, USA). Cells were maintained in a humidified environment at 37°C with 5% CO2 in air.

Four different human breast cancer cell lines (MCF-7, T47D, MDA-MB-231 and MBA-MB-435) purchased from the American Type Culture Collection (Manassas, VA, USA) were grown and maintained in appropriate growth media; minimal essential medium for MCF-7 and RPMI 1640 for T47D, MDA-MB-231 and MBA-MB-435 (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Welgene, Daegu, Korea). L-glutamine, penicillin, streptomycin and gentamicin (Life Technologies Korea, LLC, Seoul, Korea) were used at the usual concentrations. For all experiments, breast cancer cells were harvested by trypsinization (0.25% trypsin and 0.02% EDTA; Life Technologies Korea, LLC), seeded and grown in the appropriate media containing 10% FBS in a humidified 95% air 5% CO2 atmosphere.

Cell growth rate measurements. The CellTiter 96® AQ<sub>Green</sub> Non-Radioactive Cell Proliferation Assay (Promega Corporation, Madison, WI, USA) was used for the measurement of cell growth rate in breast cancer cells according to the manufacturer's instructions. The CellTiter 96® AQ<sub>Green</sub> Assay is composed of solutions of a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine methosulfate; PMS).

Briefly, HMECs, MCF-7 and T47D (1,000 cells/well) were plated in 96-well plates. Following a 24 h resting period, LGD1069, LG100268 and Ro25-7386 were added into the growth media and cell culture continued for 8-12 days. Each measurement day (every 2 days), MTS (Promega Corporation) was added to the cells (20 µl combined MTS/PMS solution per 100 µl culture medium) and further incubation was conducted for 2 h. MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm was measured directly using an ELISA plate reader (Gemini EM Microplate reader, Versa Max, Fluorescence readers; Molecular Devices, Sunnyvale, CA, USA). Each data point was performed in quadruplicate and the results were presented as the mean absorption (optical density).

RNA target preparation/Affymetrix microarray analysis. Total RNA was extracted from different breast cells treated with retinoids using the guanidinium isothiocyanate method (TRIZol reagent; Invitrogen Life Technologies) followed by purification using an RNeasy column (Qiagen, Valencia, CA, USA). RNA quality was assessed using the 2100 Bioanalyzer Instrument (Agilent Technologies, Inc., Palo Alto, CA, USA). A total of 10 µg total RNA was processed for use on the microarray using the Affymetrix GeneChip One-Cycle Target Labeling kit (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. The resultant biotinylated cRNA was fragmented and then hybridized to the Affymetrix U133 Plus 2.0 GeneChip. The arrays were washed, stained and scanned using the Affymetrix 450 Fluidics Station and GeneChip Scanner 3000 7G (Affymetrix, Inc.) according to the manufacturer's recommendations. Expression values were generated using Microarray Suite software, version 5.0 (Affymetrix, Inc.).
Table I. Forward and reverse primers for amplification of targeted genes with reverse transcription-quantitative polymerase chain reaction.

| Target gene               | Forward primer                        | Reverse primer                        |
|---------------------------|---------------------------------------|---------------------------------------|
| BAX                       | 5'-TGGAGGCTGACAGAGATGATTG-3'          | 5'-GAAGTTGCCGTCAAGAAACATG-3'          |
| E-cadherin                | 5'-CAGTGCCACTGGCTGAG-3'               | 5'-GGTGTAGCTCAGCAGTAAAG-3'            |
| FOXO3A                    | 5'-TCAATCGAGAATCTGCACCA-3'            | 5'-GAATCTCTCAAGCCCATGGT-3'            |
| Integrin α6               | 5'-TTTCCCCATTTCTTCTTGAAGTT-3'         | 5'-GGAAAGAAAGTGAACCTTGGAGCA-3'        |
| Integrin β4               | 5'-TTCCAATCAGACAGGAGAC-3              | 5'-CTTGAGGTTGTCCAGATCAT-3'            |
| PXN                       | 5'-TGCGCTCGCTGCTCGATATTTC-3'          | 5'-GTCAGGCTGTCACCCACTTATCC-3'         |
| PTEN                      | 5'-AGACGCTGCAAGATAAAGGAGA-3           | 5'-GGATCACAGCTAGTGGTGCAG-3'           |
| STAT                      | 5'-CTGCTGCGGTTACGTGAGAGA-3            | 5'-CCAAGTGAAGTGACCCCTCC-3'            |
| Collagen type VI α3       | 5'-CTGGGCCGACAGATACACCATGTG-3'        | 5'-GCAAGTTCTCTCTGGCTTCG-3'            |

software (Agilent Technologies) for gene expression. Fold change values for genes were calculated as the ratio of the signal values of the experimental group compared with the control group. Alterations in gene expression >2-fold were considered to be statistically significant. Genes of interest were selected by referring to the PathArt program which shows intersection of genes in several signaling pathways.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Cells were cultured to 80-90% confluence. Total RNA was prepared using the QiaGen RNeasy Mini kit (Qiagen). The RT reaction was performed using 1 µg total RNA which was reverse-transcribed into cDNA using a random hexamer primer (GeneAmp RNA PCR Core kit; Applied Biosystems Life Technologies, Foster City, CA, USA), according to the manufacturer's instructions. cDNA of the 7 selected genes and an internal reference gene (GAPDH) was produced from each sample and was quantified using a fluorescence-based real-time detection method (iCycler; Bio-Rad Laboratories, Inc., Hercules, CA, USA). RT-qPCR analysis was performed using the standard methods recommended by the RT-qPCR kit supplier (SYBR® Green Dye-Based Gene Expression Detection; Applied Biosystems Life Technologies). Primer sequences used for detection of RXRα-regulated genes are shown in Table I (Cosmo Genetech, Seoul, Korea). For the endogenous control, human GAPDH labeled with VIC™ dye provided by Applied Biosystems Life Technologies was used. The amplification conditions were as follows: 30 sec at 95°C and 3 min at 95°C, and 30 sec at 95°C and 60 sec at 65°C for 40 cycles, followed by a final extension for 20 min at 72°C. The ratio between the values obtained provided the relative gene expression levels.

Western blot analysis. Whole cell extracts were prepared using 1X sodium dodecyl sulfate (SDS) laemmlili lysis buffer (125 mM Tris-HCl, pH 6.8; 1% SDS; 2% β-mercaptoethanol). Total cell lysates with equal quantities of protein (30 µg) were subjected to 10% SDS-PAGE and subsequently electrotransferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Inc.). Membranes were blocked with 5% skimmed milk in PBST (phosphate-buffered saline containing 0.1% Tween 20) for 1 h at room temperature, then incubated overnight with primary antibodies in PBST containing 2.5% bovine serum albumin (1:1,000 dilution). Subsequent to washing with PBST, the blot was further incubated for 1 h at room temperature with peroxidase conjugated anti-rabbit or anti-mouse antibodies (Pierce Technology Corporation, Holmdel, NJ, USA) in PBST and then visualized using the enhanced chemiluminescence system (GE Healthcare Life Sciences, Chalfont, UK). Protein expression was normalized using β-actin expression.

Statistical analysis. All experiments were performed in triplicate. Statistical analyses were performed using Microsoft Excel 2007 (Microsoft Corporation, Albuquerque, NM, USA). The data for the MTS assay and RT-qPCR are expressed as the mean ± standard deviation. Student's t-test was used for single variable comparisons, and P<0.05 was considered to indicate a statistically significant difference.

Results

Anti-proliferative activity of rexinoids. In Fig. 1, the structures of LGD1069 and LG100268 are presented. The anti-proliferative effects of rexinoids in normal and malignant breast cells were investigated. It was observed that LGD1069 and LG100268 significantly suppressed cell growth in HMECs at 10 µM; whereas co-treatment with LGD1069 and LG100268 reduced cell growth at 1 and 10 µM suggesting that these two rexinoids possess the capacity to prevent mammary cell growth (Fig. 2). By contrast, LGD1069 weakly (10 µM, P<0.05) inhibited cell growth in MCF-7 cells while the compound strongly and significantly suppressed cell growth in a dose-dependent manner in T47D cells (0.1 µM, P<0.01; 1 and 10 µM, P<0.001) (Fig. 3). Notably, LGD1069 induced mild inhibition (P<0.05) of cell growth in MDA-MB-231 cells at 10 µM while rexinoids did not affect cell growth in MDA-MB-435 cells (Fig. 3). This result indicates that LGD1069 is able to inhibit the growth of ER-negative breast cancer with therapeutic potency.

In addition, Ro25-7386, the RXRα agonist significantly suppressed cell growth in a dose-dependent manner in HMECs. Ro25-7386 strongly reduced T47D cell growth at 1 µM and induced suppression of cell growth in MCF-7 cells at day 8 at 1 µM (Fig. 4). These results suggest that RXRα is
important in the suppression of growth induced by rexinoids in breast cells.

Expression of RXRα in breast cells. The RXRα level in normal and malignant breast cells was next determined. It was observed that all breast cell lines express RXRα but with different intensities. MCF-7 and T47D expressed higher levels of RXRα (Fig. 5). Notably, the ER-negative breast cancer cell lines, MDA-MB-231 and MDA-MB-435, also expressed RXRα.

Identification of target genes regulated by rexinoids in normal and malignant breast cells by Affymetrix microarray. Finally, the genes regulated by rexinoids in normal (HMECs) and
Table II. Genes up- and downregulated by Ro25-7386 in human mammary epithelial cells.

A. Genes upregulated by Ro25-7386

| Probe set | Gene                                                                 | Fold change |
|-----------|----------------------------------------------------------------------|-------------|
| 213872_at | gb:BE465032/DB_XREF=gi:9510807/DB_XREF=gv76g09.x1/CLONE=IMAGE:3179392/FEA=EST/CNT=34/TID=Hs.173685.1/TIER=Stack/STK=15/UG=Hs.173685/LL=81688/UG_GENE=FLJ12619/UG_TITLE=hypothetical protein FLJ12619 | 27.55       |
| 204989_s_at | Integrin, β4                                                        | 26.60       |
| 210317_s_at | Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, epsilon polypeptide | 22.14       |
| 200935_at | Calreticulin                                                        | 21.26       |
| 201130_s_at | Cadherin 1, type 1, E-cadherin (epithelial)                        | 20.66       |
| 201123_s_at | Eukaryotic translation initiation factor 5A                        | 19.04       |
| 200751_s_at | Heterogeneous nuclear ribonucleoprotein C (C1/C2)                  | 18.97       |
| 214007_s_at | PTK9 protein tyrosine kinase 9                                      | 17.37       |
| 203392_s_at | C-terminal binding protein 1                                        | 16.35       |
| 204427_s_at | Coated vesicle membrane protein                                     | 16.00       |
| 216971_s_at | Plectin 1, intermediate filament binding protein 500 kDa           | 15.17       |
| 217211_at | Consensus includes gb:DS0604/DEF=Human β-cytoplasmic actin (ACTBP9) pseudogene/FEA=CDS/DB_XREF=gi:2094759/UG=Hs.248007 Human β-cytoplasmic actin (ACTBP9) pseudogene | 14.35       |
| 215780_s_at | SET translocation (myeloid leukemia-associated)                     | 12.51       |
| 201971_s_at | ATPase, H+ transporting, lysosomal 70 kDa, V1 subunit A             | 11.75       |
| 204426_s_at | Coated vesicle membrane protein                                     | 11.74       |
| 220494_s_at | gb:NM_018678.1/DEF=Homo sapiens lipopolysaccharide specific response-68 protein (LSR68), mRNA./FEA=mRNA/GEN=LSR68/PROD=lipopolysaccharide specific response-68 protein/DB_XREF=gi:8923914/UG=Hs.103189 lipopolysaccharide specific response-68 protein/ | 11.18       |
| 215177_s_at | Integrin, α6                                                        | 10.97       |
| 214693_s_at | Hypothetical protein MGC8902///AG1///hypothetical protein DJ328E19.C1.1///hypothetical protein LOC200030///hypothetical protein LOC348482 | 10.34       |
| 211905_s_at | Integrin, β4                                                        | 10.34       |
| 201048_s_at | RAB6A, member RAS oncogene family                                   | 10.03       |
| 214701_s_at | Fibronectin 1                                                       | 10.01       |
| 210092_at | Mago-nashi homolog, proliferation-associated (Drosophila)           | 9.74        |
| 212107_s_at | DEAH (Asp-Glu-Ala-His) box polypeptide 9                            | 9.68        |
| 202118_s_at | Copine III                                                          | 9.48        |
| 217234_s_at | Villin 2 (ezrin)                                                    | 9.09        |
| 208853_s_at | Calnexin                                                            | 7.59        |
| 201742_s_at | Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor) | 7.44        |
| 208750_s_at | ADP-ribosylation factor 1                                           | 7.31        |
| 203803_at | Prenylcysteine oxidase 1                                            | 7.31        |
| 211162_s_at | Stearoyl-CoA desaturase (δ-9-desaturase)                            | 7.30        |
| 202856_s_at | Solute carrier family 16 (monocarboxylic acid transporters), member 3 | 7.26        |
| 200796_s_at | Myeloid cell leukemia sequence 1 (BCL2-related)                     | 7.25        |
| 213606_s_at | Rho GDP dissociation inhibitor (GDI) α                              | 7.25        |
| 201373_s_at | Plectin 1, intermediate filament binding protein 500kDa             | 7.19        |
| 208057_s_at | GLI-Kruppel family member GLI2                                      | 7.04        |
| 217294_s_at | Enolase 1, (α)                                                      | 6.99        |
| 213875_s_at | Chromosome 6 open reading frame 62                                  | 6.93        |
| 91816_f_at | Ring finger and KH domain containing 1                              | 6.90        |
| 200806_s_at | Heat shock 60 kDa protein 1                                         | 6.69        |
| 214845_s_at | Calumenin                                                           | 6.66        |
| 211823_s_at | Paxillin                                                            | 5.75        |
| 206665_s_at | BCL2-like 1                                                         | 5.40        |
Table II. Continued.

| Probe set | Gene                                      | Fold change |
|-----------|-------------------------------------------|-------------|
| 208637_s_at | Actinin, α1                              | 5.11        |
| 208677_s_at | Basigin (OK blood group)                 | 4.66        |
| 221499_s_at | Syntaxin 16                               | 4.16        |
| 209226_s_at | Transportin 1                             | 3.90        |
| 201752_s_at | Adducin 3 (γ)                            | 3.90        |
| 200766_at | Cathepsin D (lysosomal aspartyl protease) | 3.90        |
| 203085_s_at | Transforming growth factor, β1            | 3.75        |
| 211833_s_at | BCL2-associated X protein                | 3.65        |
| 208852_s_at | Calnexin                                | 3.49        |
| 210655_s_at | Forkhead box O3A                      | 3.33        |

B, Genes downregulated by Ro25-7386

| Probe set | Gene                                      | Fold change |
|-----------|-------------------------------------------|-------------|
| 203991_s_at | Ubiquitously transcribed tetratricopeptide repeat, X chromosome | -5.32       |
| 200568_at | gb:NM_018582.1/DEF=Homo sapiens hypothetical protein PRO1483 (PRO1483), mRNA/FEA=mRNA/GEN=PRO1483/PROD=hypothetical protein PRO1483/DB_XREF=gi:8924047/UG=Hs.279694 hypothetical protein PRO1483/FL=gb:AF116635.1 gb:NM_018582.1 | -4.72       |
| 213705_at | Methionine adenosyltransferase II, α     | -4.64       |
| 201438_at | Collagen, type VI, α3                   | -4.59       |
| 217665_at | Consensus includes gb:AA420614/FEA=EST/DB_XREF=gi:2094586/DB_XREF= est: nc62g02.x1/CLONE=IMAGE:745874/UG=Hs.188826 ESTs, Moderately similar to G02654 ribosomal protein L39 H. sapiens | -4.17       |
| 209459_s_at | 4-aminobutyrate aminotransferase       | -3.99       |
| 220992_s_at | Chromosome 1 open reading frame 25///chromosome 1 open reading frame 25 | -3.81       |
| 222294_s_at | Eukaryotic translation initiation factor 2C, 2  | -3.78       |
| 221995_s_at | Consensus includes gb:BF195165/FEA=EST/DB_XREF=gi:11081754/DB_XREF= est: 7n16b01.x1/CLONE=IMAGE:3564624/UG=Hs.182695 hypothetical protein MGC3243 | -3.71       |
| 215095_at | Esterase D/formylglutathione hydrolase   | -3.68       |
| 212675_s_at | KIAA0582                               | -3.66       |
| 210187_at | FK506 binding protein 1A, 12 kDa        | -3.65       |
| 204634_at | NIMA (never in mitosis gene a)-related kinase 4 | -3.59       |
| 203791_at | Dmx-like 1                              | -3.53       |
| 205583_s_at | Chromosome X open reading frame 45     | -3.53       |
| 218352_at | Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1 | -3.52       |
| 209788_s_at | Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator | -3.48       |
| 212959_s_at | MGC4170 protein                      | -3.47       |
| 205802_at | Transient receptor potential cation channel, subfamily C, member 1 | -3.43       |
| 207323_at | Protein kinase (cAMP-dependent, catalytic) inhibitor γ | -3.40       |
| 202149_at | Neural precursor cell expressed, developmentally downregulated 9 | -3.39       |
| 213225_at | Protein phosphatase 1B (formerly 2C), magnesium-dependent, β isoform | -3.39       |
| 213624_at | Sphingomyelin phosphodiesterase, acid-like 3A | -3.39       |
| 207855_s_at | Mid-1-related chloride channel 1         | -3.37       |
| 204415_at | Interferon, α-inducible protein (clone IFI-6-16) | -3.29       |
| 210017_at | Mucosa associated lymphoid tissue lymphoma translocation gene 1 | -3.12       |
| 205420_at | Peroxisomal biogenesis factor 7         | -3.05       |
| 219317_at | Polymerase (DNA directed) iota          | -3.01       |
| 204176_at | Kelch-like ECT2 interacting protein      | -3.00       |
| 203741_s_at | Adenylate cyclase 7                    | -2.95       |
| 205034_at | Cyclin E2                               | -2.94       |
| 204078_at | Synaptonemal complex protein SC65       | -2.90       |
malignant (MCF-7, T47D and MDA-MB-231) breast cells were identified. Gene expression profiles were established using the Affymetrix microarray (human genome U133A 2.0). Among them, several genes involved in cell death, cell growth/maintenance, signal transduction and response to stimulus were identified.

In HMECs, 638 genes upregulated and 347 genes downregulated by Ro25-7386 with alterations in fold induction >2-fold were identified. A total of 22 genes were strongly upregulated (>10-fold) and 5 genes were strongly downregulated (>4-fold) in expression levels by Ro25-7386 (Table IIA and B). Among them, several genes were notable, including integrin β4, E-cadherin (CDH1), C-terminal binding protein 1 (CtBP1), integrin α6, paxillin (PAX), BAX, forkhead box O3A (FOXO3A) and signal transducer and activator of transcription 3 (STAT3) (upregulated genes), and collagen type VI α3 and cell division cycle 42 (CDC42) (downregulated genes).

In MCF-7 cells, 83 genes were upregulated and 98 genes downregulated by Ro25-7328 with alterations in fold induction >2-fold were identified (Table III). Among them, several genes were recognized including transforming growth factor β2, immunoglobulin heavy constant γ1, protein kinase Cδ binding protein, interleukin 6 receptor and neurophilin 2 (upregulated genes), and cathepsin S, zinc finger protein 36, integrin β4, transforming growth factor β1, PAX and CtBP1 (downregulated genes).

In T47D cells, 16 upregulated genes and 3 downregulated genes modulated by LGD1069 were observed (Table IV), whereas 3 upregulated genes and 5 downregulated genes

---

Table II. Continued.

| Probe set       | Gene                                                   | Fold change |
|-----------------|--------------------------------------------------------|-------------|
| 203881_s_at     | Dystrophin (muscular dystrophy, Duchenne and Becker types) | -2.88       |
| 209717_at       | Ecotropic viral integration site 5                    | -2.87       |
| 213473_at       | BRCA1 associated protein                               | -2.86       |
| 215949_x_at     | Immunoglobulin heavy constant µ                        | -2.83       |
| 205668_at       | Lymphocyte antigen 75                                  | -2.83       |
| 219688_at       | Bardet-Biedl syndrome 7                                | -2.82       |
| 207845_s_at     | Anaphase promoting complex subunit 10                  | -2.80       |
| 208920_at       | Sorcin                                                 | -2.79       |
| 218002_s_at     | Chemokine (C-X-C motif) ligand 14                      | -2.53       |
| 208727_s_at     | Cell division cycle 42 (GTP binding protein, 25 kDa)   | -2.25       |

Figure 4. Effect of Ro25-7386 on the growth of breast cells. HMECs, MCF-7 and T47D cells were treated with Ro25-7386 for 0-12 days. The relative cell growth rate was measured by an MTS assay. Data are presented as the mean of three independent experiments (error bars denote the standard deviation; *P<0.05, **P<0.01 and ***P<0.001 vs. control). DMSO, dimethyl sulfoxide; OD, optical density.
Table III. Genes up- and downregulated by Ro25-7386 in MCF-7 cells.

A, Genes upregulated by Ro25-7386

| Probe set | Gene                                                                 | Fold change |
|-----------|----------------------------------------------------------------------|-------------|
| 209909_s_at | Transforming growth factor, β2                                      | 4.94        |
| 211430_s_at | Immunoglobulin heavy constant γ 1 (G1m marker)                      | 3.82        |
| 213010_at  | Protein kinase C, δ binding protein                                 | 3.76        |
| 63825_at   | Abhydrolase domain containing 2                                     | 3.40        |
| 208993_s_at | Peptidyl-prolyl isomerase G (cyclophilin G)                        | 3.39        |
| 204681_s_at | Rap guanine nucleotide exchange factor (GEF) 5                     | 3.26        |
| 213536_s_at | gb:AA910614/DB_XREF=gi:3049004/DB_XREF=ok61b04.s1/CLONE=IMAGE:1518415/FEA=EST/CNT=42/TID=Hs.842852.2/TIER=Stack/STK=12/UG=Hs.84285/LL=7329/UG_GENE=UBE2I/UG_TITLE=ubiquitin-conjugating enzyme E2I (homologous to yeast UBC9) | 3.20        |
| 213087_s_at | Eukaryotic translation elongation factor 1 δ (guanine nucleotide exchange protein) | 3.09        |
| 213747_at  | Consensus includes gb:AA910371/FEA=EST/DB_XREF=gi:3049661/DB_XREF=est:zf50b09.s1/CLONE=IMAGE:380345/UG=Hs.223014 antizyme inhibitor | 2.99        |
| 210136_at  | Myelin basic protein                                                | 2.88        |
| 212952_at  | Butyrobetaine (γ), 2-oxoglutarate dioxygenase (γ-butyrobetaine hydroxylase) 1 | 2.92        |
| 212455_at  | ATPase, class V, type 10A                                           | 2.87        |
| 213789_at  | Consensus includes gb:N58493/FEA=EST/DB_XREF=gi:1202383/DB_XREF=est:yv72d01.s1/CLONE=IMAGE:248257/UG=Hs.75105 emopamil-binding protein (sterol isomerase) | 2.86        |
| 217464_at  | Consensus includes gb:L48784/DEF=050 Homo sapiens cDNA/FEA=mRNA/DB_XREF=gi:1066715/UG=Hs.182426 ribosomal protein S2 | 2.83        |
| 210841_s_at | Neuropeptin 2                                                       | 2.82        |
| 204378_at  | Breast carcinoma amplified sequence 1                              | 2.80        |
| 208859_s_at | α thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | 2.76        |
| 221018_s_at | Tudor domain containing 1/tudor domain containing 1                | 2.76        |
| 218876_at  | Brain specific protein//brain specific protein                      | 2.73        |
| 215081_at  | KIAA1024 protein                                                   | 2.71        |
| 201510_at  | E74-like factor 3 (ets domain transcription factor, epithelial-specific ) | 2.69        |
| 210089_s_at | Laminin, α4                                                       | 2.68        |
| 218859_s_at | Chromosome 20 open reading frame 6                                  | 2.65        |
| 211626_s_at | v-ets erythroblastosis virus E2 oncogene like (avian)//v-ets erythroblastosis virus E2 oncogene like (avian) | 2.64        |
| 214316_s_at | gb:AI378706/DB_XREF=gi:4188559/DB_XREF=tb91f09.x1/CLONE=IMAGE:2061737/FEA=EST/CNT=13/TID=Hs.16488.3/TIER=Stack/STK=13/UG=Hs.16488/LL=811/UG_GENE=CALR/UG_TITLE=calreticulin | 2.64        |
| 220657_at  | Kelch-like 11 (Drosophila)                                         | 2.61        |
| 206490_at  | Discs, large (Drosophila) homolog-associated protein 1             | 2.60        |
| 208383_s_at | Phosphoenolpyruvate carboxykinase 1 (soluble)                      | 2.59        |
| 214884_at  | gb:AL033403/DB_XREF=gi:3859054/FEA=mRNA/CNT=15/TID=Hs.89543.1/TIER=ConsEnd/STK=0/UG=Hs.89543/LL=4168/UG_GENE=MCF2/UG_TITLE=MCF2 cell line derived transforming sequence/DEF=Human DNA sequence from clone 88D7 on chromosome Xq25-26.3 Contains F9 (coagulation factor IX (plasma thromboplastic component, Christmas disease, haemophilia B)), dbl oncogene. EST, STS, GSS | 2.59        |
| 201506_at  | Transforming growth factor, β-induced, 68 kDa                     | 2.18        |
| 213979_s_at | Consensus includes gb:BF894434/FEA=EST/DB_XREF=gi:12387246/DB_XREF=est:602307971F1/CLONE=IMAGE:4399313/UG=Hs.239737 C-terminal binding protein 1 | 2.50        |
### Table III. Continued.

| Probe set   | Gene                                                                 | Fold change |
|-------------|----------------------------------------------------------------------|-------------|
| 211253_x_at | Peptide YY                                                           | 2.38        |
| 206879_s_at | Neuregulin 2                                                         | 2.33        |
| 208835_s_at | Cisplatin resistance-associated overexpressed protein               | 2.33        |
| 201506_at  | Transforming growth factor, β-induced, 68 kDa                       | 2.18        |

### B. Genes downregulated by Ro25-7386

| Probe set   | Gene                                                                 | Fold change |
|-------------|----------------------------------------------------------------------|-------------|
| 202901_x_at | Cathepsin S                                                          | -64.37      |
| 201367_s_at | Zinc finger protein 36, C3H type-like 2                              | -5.67       |
| 213606_s_at | Rho GDP dissociation inhibitor (GDI) α                               | -5.01       |
| 211136_s_at | Cleft lip and palate associated transmembrane protein 1             | -4.59       |
| 204989_s_at | Integrin, β4                                                         | -4.51       |
| 213042_s_at | ATPase, Ca++ transporting, ubiquitous                                | -4.42       |
| 216971_s_at | Plectin 1, intermediate filament binding protein 500 kDa            | -4.37       |
| 201167_x_at | Rho GDP dissociation inhibitor (GDI) α                               | -4.14       |
| 219529_at  | Chloride intracellular channel 3                                     | -3.97       |
| 218813_s_at | SH3-domain GRB2-like endophilin B2                                   | -3.93       |
| 211905_s_at | Integrin, β4                                                         | -3.87       |
| 211672_s_at | Actin related protein 2/3 complex, subunit 4, 20 kDa/actin related protein 2/3 complex, subunit 4, 20 kDa | -3.70       |
| 207521_s_at | ATPase, Ca++ transporting, ubiquitous                                | -3.44       |
| 213986_s_at | Chromosome 19 open reading frame 6                                   | -3.43       |
| 207824_s_at | MYC-associated zinc finger protein (purine-binding transcription factor) | -3.42       |
| 203085_s_at | Transforming growth factor, β1 (Camurati-Engelmann disease)          | -3.34       |
| 203953_s_at | Claudin 3                                                            | -3.26       |
| 211019_s_at | Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)           | -3.22       |
| 209872_s_at | Plakophilin 3                                                        | -3.20       |
| 214326_x_at | Jun D proto-oncogene                                                 | -3.14       |
| 208677_s_at | Basigin (OK blood group)                                             | -3.12       |
| 201245_s_at | OTU domain, ubiquitin aldehyde binding 1                            | -3.08       |
| 203751_x_at | Jun D proto-oncogene                                                 | -3.08       |
| 203370_s_at | PDZ and LIM domain 7 (enigma)                                        | -3.05       |
| 203028_s_at | Cytochrome b-245, α polypeptide                                      | -3.02       |
| 210954_s_at | KIAA0669 gene product                                                | -2.99       |
| 211823_s_at | Paxillin                                                             | -2.97       |
| 200968_s_at | Peptidylprolyl isomerase B (cyclophilin B)                           | -2.93       |
| 205463_s_at | Platelet-derived growth factor α polypeptide                         | -2.87       |
| 210317_s_at | Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ε polypeptide | -2.87       |
| 211300_s_at | Tumor protein p53 (Li-Fraumeni syndrome)                             | -2.84       |
| 214251_s_at | Nuclear mitotic apparatus protein 1                                  | -2.81       |
| 207722_s_at | BTB (POZ) domain containing 2                                        | -2.80       |
| 216969_s_at | Kinesin family member 22                                             | -2.79       |
| 203809_s_at | v-akt murine thymoma viral oncogene homolog 2                        | -2.76       |
| 218848_at  | Hypothetical protein MGC2655                                         | -2.73       |
| 212090_at  | Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding) | -2.69       |
| 201373_at  | Plectin 1, intermediate filament binding protein 500 kDa            | -2.68       |
| 218302_at  | Presenilin enhancer 2                                                | -2.68       |
| 213887_s_at | Polymerase (RNA) II (DNA directed) polypeptide E, 25 kDa             | -2.67       |
| 201369_s_at | Zinc finger protein 36, C3H type-like 2                              | -2.67       |
were identified to be modulated by LG100268 (Table V) with alterations in fold induction >2-fold. According to the data, several notable genes induced by LGD1069 and LG100268 in T47D cells were identified, including cytochrome P450, dehydrogenase/reductase member 3, metallothionein, neuro-oncological ventral antigen 1 and regulator of G-protein signaling 1 (for LGD1069), and chemokine, glutamate receptor, colon carcinoma-related protein and insulin-like growth factor binding protein 7 (for LG100268). In addition, 3 upregulated genes and 5 downregulated genes by Ro25-7386 were identified with alterations in fold induction >2-fold in T47D cells. Among them, chemokine (upregulated genes), and glutamate receptor, ionotropic kainite 2, colon carcinoma-related protein, insulin-like growth factor binding protein 7 and growth differentiation factor 8 were identified (Table VI).

Table IV. Genes up- and downregulated by LGD1069 in T47D cells.

A, Genes upregulated by LGD1069

| Probe set     | Gene                                                   | Fold change |
|---------------|--------------------------------------------------------|-------------|
| 204326_s_at   | Metallothionein 1G                                     | 2.19        |
| 206461_x_at   | Metallothionein 1H                                     | 2.15        |
| 204470_at     | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α) | 2.14        |
| 204745_x_at   | Metallothionein 1G                                     | 2.13        |
| 217028_at     | Chemokine (C-X-C motif) receptor 4                     | 2.04        |
| 203392_s_at   | C-terminal binding protein 1                           | -2.50       |
| 200796_s_at   | Myeloid cell leukemia sequence 1 (BCL2-related)        | -2.43       |
| 206665_s_at   | BCL2-like                                              | -2.35       |

Table III. Continued.

| Probe set     | Gene                                                   | Fold change |
|---------------|--------------------------------------------------------|-------------|
| 203392_s_at   | C-terminal binding protein 1                           | -2.50       |
| 200796_s_at   | Myeloid cell leukemia sequence 1 (BCL2-related)        | -2.43       |
| 206665_s_at   | BCL2-like                                              | -2.35       |
In MDA-MB-231 cells, a total of 335 upregulated genes and 320 downregulated genes modulated by LGD1069 were observed (Table VII); whereas 118 upregulated genes and 432 downregulated genes were modulated by LGD100268 with alterations in fold induction >2-fold. According to the data, several notable genes were identified, including several types of hypothetical protein, zinc finger homeobox 1b, recombination activating gene 2 and tumor protein D52 (for LGD1069), and zinc finger protein 21, Mdm2, and gonadotropin-releasing hormone 1 (for LG100268).

Table V. Genes up- and downregulated by Ro25-7386 in T47D cells.

| Probe set | Gene                                                                 | Fold change |
|-----------|-----------------------------------------------------------------------|-------------|
| A, Genes upregulated by Ro25-7386 |
| 215653_at  | Consensus includes gb:AF339805.1/DEF=Homo sapiens clone IMAGE:248602, mRNA sequence /FEA=mRNA/DB_XREF=gi:13507343/UG=Hs.326719 Homo sapiens clone IMAGE:248602, mRNA sequence | 4.74        |
| 215924_at  | Consensus includes gb:AK022102.1/DEF=Homo sapiens cDNA FLJ12040 fis, clone HEMBB1001944 /FEA=mRNA/DB_XREF=gi:10433423/UG=Hs.296687 Homo sapiens cDNA FLJ12040 fis, clone HEMBB1001944 | 2.87        |
| 204470_at  | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α) | 2.26        |
| B, Genes downregulated by Ro25-7386 |
| 215655_at  | Glutamate receptor, ionotropic, kainate 2                             | -3.29       |
| 220327_at  | Colon carcinoma-related protein                                       | -2.94       |
| 213910_at  | Insulin-like growth factor binding protein 7                          | -2.66       |
| 207145_at  | Growth differentiation factor 8                                       | -2.49       |
| 210806_at  | KIAA0998                                                             | -2.30       |

Table VI. Genes up- and downregulated by LG100268 in T47D cells.

| Probe set | Gene                                                                 | Fold change |
|-----------|-----------------------------------------------------------------------|-------------|
| A, Genes upregulated by LG100268 |
| 215653_at  | Consensus includes gb:AF339805.1/DEF=Homo sapiens clone IMAGE:248602, mRNA sequence /FEA=mRNA/DB_XREF=gi:13507343/UG=Hs.326719 Homo sapiens clone IMAGE:248602, mRNA sequence | 4.74        |
| 215924_at  | Consensus includes gb:AK022102.1/DEF=Homo sapiens cDNA FLJ12040 fis, clone HEMBB1001944 /FEA=mRNA/DB_XREF=gi:10433423/UG=Hs.296687 Homo sapiens cDNA FLJ12040 fis, clone HEMBB1001944 | 2.87        |
| 204470_at  | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α) | 2.26        |
| B, Genes downregulated by LG100268 |
| 215655_at  | Glutamate receptor, ionotropic, kainate 2                             | -3.29       |
| 220327_at  | Colon carcinoma-related protein                                       | -2.94       |
| 213910_at  | Insulin-like growth factor binding protein 7                          | -2.66       |
| 207145_at  | Growth differentiation factor 8                                       | -2.49       |
| 210806_at  | KIAA0998                                                             | -2.30       |

In MDA-MB-231 cells, a total of 335 upregulated genes and 320 downregulated genes modulated by LGD1069 were observed (Table VII); whereas 118 upregulated genes and 432 downregulated genes were modulated by LGD100268 (Table VIII) with alterations in fold induction >2-fold. According to the data, several notable genes were identified, including several types of hypothetical protein, zinc finger homeobox 1b, recombination activating gene 2 and tumor protein D52 (for LGD1069), and zinc finger protein 21, Mdm2, and gonadotropin-releasing hormone 1 (for LG100268).

Confirmation of the alterations of modulation of RXRα target genes of HMECs by RT-qPCR and western blot analysis. The induction of a total of 7 genes by rexinoid (mRNA levels) was confirmed by RT-qPCR assays. These 7 genes are as follows: Integrin β4, integrin α6, CDH1, PAX, BAX, FOXO3A and...
Table VII. Genes up- and downregulated by LGD1069 in MDA-MB-231.

| Probe set | Gene | Fold change |
|-----------|------|-------------|
| 219948_x_at | Hypothetical protein FLJ21934 | 232.43 |
| 209672_s_at | Hypothetical protein FLJ20323 | 69.61 |
| 207750_at | gb:NM_018510.1/DEF=Homo sapiens hypothetical protein PRO1866 (PRO1866), mRNA. | 30.50 |
| 203603_s_at | Zinc finger homeobox 1b | 10.18 |
| 217698_at | Consensus includes gb:AV651668/FEA=EST/DB_XREF=gi:9872682/DB_XREF=est:AV651668/CLONE=GLCCSC04/UG=Hs.282480 ESTs | 10.11 |
| AFFX-r2- | E. coli/GEN=biob/DB_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides | 9.76 |
| BioB-M_at | 2482-2739 of gb:J04423.1/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiotin | 9.65 |
| 205386_s_at | Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) | 9.42 |
| 216119_s_at | Chromosome 20 open reading frame 28 | 9.42 |
| AFFX- | E. coli/GEN=biob/DB_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides | 9.32 |
| BioB-M_at | 2482-2739 of gb:J04423.1/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiotin | 9.32 |
| 209613_s_at | Alcohol dehydrogenase IB (class I), β polypeptide | 8.85 |
| Ec-bio-B_3-at | 2772-3004 of gb:J04423.1/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiotin | 8.78 |
| 217194_at | Consensus includes gb:AB007970.1/DEF=Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501. | 7.08 |
| 205524_s_at | Hylauronan and proteoglycan link protein 1 | 7.06 |
| 215514_at | Consensus includes gb:AL080072.1/DEF=Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616)/FEA=mRNA/DB_XREF=gi:5262482/UG=Hs.21195 Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616) | 6.85 |
| 214774_x_at | Trinucleotide repeat containing 9 | 6.70 |
| 215526_at | Consensus includes gb:AL050145.1/DEF=Homo sapiens mRNA; cDNA DKFZp586C2020 (from clone DKFZp586C2020)/FEA=mRNA/DB_XREF=gi:5268436/UG=Hs.225986 Homo sapiens mRNA; cDNA DKFZp586C2020 (from clone DKFZp586C2020) | 6.22 |
| 211091_s_at | Neurofibromin 2 (bilateral acoustic neuroma) | 6.21 |
| 221959_at | Hypothetical protein MGC39325 | 6.11 |
| 206863_s_at | gb:U76376.1/DB_XREF=gi:1923234/GEN=HRK/FEA=FLmRNA/CNT=9/TID=Hs.87247/0/TIER=ConsEnd/STK=0/UG=Hs.87247/LL=8739/DEF=Homo sapiens activator of apoptosis Hrk (HRK) mRNA, complete cds./PROD=activator of apoptosis Hrk/FL=gb:NM_003806.1/ gb:U76376.1 | 6.09 |
| 206202_at | Mesenchyme homeo box 2 (growth arrest-specific homeo box) | 5.75 |
| 205288_at | CDC14 cell division cycle 14 homolog A (S. cerevisiae) | 5.62 |
| 220931_at | Hypothetical protein MGC5590 | 5.40 |
| 216795_at | CDNA: FLJ23194 fis, clone RECO00490 | 5.29 |
| 206410_at | Nuclear receptor subfamily 0, group B, member 2 | 5.23 |
| 207647_at | Chromodomain protein, Y-linked, 1 chromodomain protein, Y-linked, 1B | 5.19 |
| 215112_s_at | MCF2 cell line derived transforming sequence-like 2 | 5.11 |
| 216775_at | Ubiquitin specific protease 53 | 4.90 |
| 220109_at | Transferrin | 4.88 |
| 217132_at | Clone 24587 mRNA sequence | 4.86 |
| 216737_at | CDNA: FLJ20872 fis, clone ADKA02604 | 4.84 |
| 220036_s_at | Lipocalin-interacting membrane receptor | 4.70 |
| AFFX-r2- | E. coli/GEN=biob/DB_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides | 4.66 |
| Ec-bio-D_3_at | 5312-5559 of gb:J04423.1, not 100% identical/DEF=E.coli 7,8-diamino-pelargonic acid (bioA),...
Table VII. Continued.

| Probe set     | Gene                                                                 | Fold change |
|---------------|----------------------------------------------------------------------|-------------|
| 220564_at     | biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein | 4.64        |
| 211611_s_at   | Tenascin XB//tenascin XB//cAMP responsive element binding protein-like 1/cAMP responsive element binding protein-like 1 | 4.61        |
| AFFX-BioDn‑3_at | E. coli/GEN=bioD/DB_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides Tenascin XB | 4.49        |
| 207272_at     | Zinc finger protein 80 (pT17)                                        | 4.49        |
| 210690_at     | Killer cell lectin-like receptor subfamily C, member 4               | 4.47        |
| 216625_at     | Consensus includes gb:AL050032.1/DEF=Homo sapiens mRNA; cDNA DKFZp566F1224 (from clone DKFZp566F1224); FEA=mRNA/DB_XREF=gi:4884272/UG=Hs.306307 Homo sapiens mRNA; cDNA DKFZp566F1224 (from clone DKFZp566F1224) | 4.37        |
| 207245_at     | UDP glycosyltransferase 2 family, polypeptide B17                    | 4.35        |
| 208014_s_at   | Neuronal thread protein AD7c-NTP                                     | 4.32        |
| 214767_s_at   | Heat shock protein, α-crystallin-related, B6                         | 4.31        |
| 216697_at     | Triple functional domain (PTPRF interacting)                         | 4.28        |
| 222341_s_at   | Consensus includes gb:AW973235/FEA=EST/DB_XREF=gi:8163081/DB_XREF=est: EST385333/UG=Hs.293697 ESTs | 4.27        |
| 207262_at     | Apolipoprotein F                                                     | 4.25        |
| 222320_at     | Consensus includes gb:AW970584/FEA=EST/DB_XREF=gi:8160429/DB_XREF=est: EST382665/UG=Hs.291033 ESTs | 4.14        |
| 206201_s_at   | Mesenchyme homeo box 2 (growth arrest-specific homeo box)            | 4.06        |
| 208019_at     | Zinc finger protein 157 (HZF22)                                      | 4.01        |
| 204991_s_at   | Neurofilibrin 2 (bilateral acoustic neuroma)                         | 3.97        |
| 207607_at     | Achaete-scute complex-like 2 (Drosophila)                            | 3.88        |
| AFFX-r2-Ec‑bioD‑5_at | E. coli/GEN=bioD/DB_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides 5024‑5244 of gb:J04423.1/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiotin | 3.83        |
| 211315_s_at   | Calcium channel, voltage-dependent, α 1G subunit                     | 3.78        |
| 205953_at     | Leucine-rich repeats and immunoglobulin-like domains 2              | 3.75        |
| 207781_s_at   | Zinc finger protein 6 (CMPX1)                                        | 3.74        |
| 216068_at     | Sodium- and chloride-activated ATP-sensitive potassium channel      | 3.69        |
| 214899_at     | Hypothetical BC331191_1                                             | 3.59        |
| 208212_s_at   | Anaplastic lymphoma kinase (Ki-1)                                    | 3.58        |

B. Genes downregulated by LGD1069

| Probe set     | Gene                                                                 | Fold change |
|---------------|----------------------------------------------------------------------|-------------|
| 215117_at     | Recombination activating gene 2                                       | -60.45      |
| 217535_at     | Consensus includes gb:AV720514/FEA=EST/DB_XREF=gi:10817666/DB_XREF=est: AV720514/CLONE=GLCGSB09/UG=Hs.282721 ESTs, Weakly similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens | -16.22      |
| 201691_s_at   | Tumor protein D52                                                    | -16.09      |
| 207674_at     | Fc fragment of IgA, receptor for                                      | -6.54       |
| 215172_at     | DKFZP566K0524 protein                                               | -5.85       |
| 218541_s_at   | Chromosome 8 open reading frame 4                                    | -5.79       |
| 215350_at     | Spectrin repeat containing, nuclear envelope 1                       | -5.69       |
| AFFX-HUMRGE/M10098_5_at | H. sapiens/GEN=18S rRNA/DB_XREF=gb:M10098.1/NOTE=SIF corresponding to nucleotides 115‑595 of gb:M10098.1/DEF=Human 18S rRNA gene, complete. | -5.59       |
| 213652_at     | Proprotein convertase subtilisin/kexin type 5                         | -5.57       |
| 216050_at     | Transcribed locus, moderately similar to NP_803425.1 DNA segment, Chr 19, | -5.43       |
Table VII. Continued.

| Probe set       | Gene                                                                 | Fold change |
|-----------------|----------------------------------------------------------------------|-------------|
| 222342_at       | Consensus includes gb:AW979196/FEA=EST/DB_XREF=gi:8170484/DB_XREF=est:EST391306/UG=Hs.292713 ESTs, Moderately similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY H.sapiens | -5.41       |
| 205638_at       | Brain-specific angiogenesis inhibitor 3                              | -5.04       |
| 217464_at       | Consensus includes gb:L487874/DEF=005 Homo sapiens cDNA/FEA=mRNA/DB_XREF=gi:1066715/UG=Hs.182426 ribosomal protein S2 | -4.97       |
| 205848_at       | Growth arrest-specific 2                                             | -4.86       |
| 206588_at       | Deleted in azzoosperma-like                                          | -4.75       |
| 213826_s_at     | Consensus includes gb:AA292281/FEA=EST/DB_XREF=gi:1940261/DB_XREF=est:zt51b03.s1/CLONE=IMAGE:725837/UG=Hs.181307 H3 histone, family 3A | -4.74       |
| 220432_s_at     | Cytochrome P450, family 39, subfamily A, polypeptide 1               | -4.48       |
| 209227_at       | Tumor suppressor candidate 3                                         | -4.41       |
| 211712_s_at     | Annexin A9/annexin A9                                                | -4.31       |
| AFFX-HUMRGE/M10098_M_at | H. sapiens/GEN=18S rRNA/DB_XREF=gb:M10098.1/NOTE=SIF corresponding to nucleotides 688-1219 of gb:M10098.1/DEF=Human 18S rRNA gene, complete. | -4.28       |
| AFFX-HUMRGE/M10098_3_at | Signal recognition particle 68 kDa                                   | -4.20       |
| 202648_at       | gb:BC0000023.1/DB_XREF=gi:12652562/FEA=FLmRNA/CNT=966/TID=Hs.298262.o/TIER=ConsEnd/STK=0/UG=Hs.298262/LL=6223/UG_GENE=RPS19/DEF=Homo sapiens, ribosomal protein S19, clone MGC:1630, mRNA, complete cds./PROD=ribosomal protein S19/FL=gb:M81757.1 g | -4.15       |
| 207815_at       | Platelet factor 4 variant 1                                          | -4.15       |
| 205363_at       | Butyrobetaine (γ), 2-oxoglutarate dioxygenase (γ-butyrobetaine hydroxylase) 1 | -4.14       |
| 213856_at       | CD47 antigen (Rh-related antigen, integrin-associated signal transducer) | -4.11       |
| 216087_at       | MRNA full length insert cDNA clone EUROIMAGE 117929                 | -4.11       |
| 211264_at       | Glutamate decarboxylase 2 (pancreatic islets and brain, 65 kDa)      | -4.03       |
| 220771_at       | Melanoma antigen                                                    | -3.83       |
| 220474_at       | Solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21 | -3.81       |
| 220281_at       | Solute carrier family 12 (sodium/potassium/chloride transporters), member 1 | -3.80       |
| 217524_x_at     | Consensus includes gb:AA018923/FEA=EST/DB_XREF=gi:1482314/DB_XREF=est:ze58d03.s1/CLONE=IMAGE:363173/UG=Hs.261204 ESTs | -3.72       |
| 211776_s_at     | Erythrocyte membrane protein band 4.1-like 3///erythrocyte membrane protein band 4.1-like 3 | -3.69       |
| 212681_at       | Erythrocyte membrane protein band 4.1-like 3                        | -3.69       |
| 217333_at       | Consensus includes gb:AL031903/DEF=Human DNA sequence from clone 1032F13 on chromosome Xq25-26.3. Contains a pseudogene similar to Keratin 18 (KRT18, Cytokeratin 18) and ESTs/FEA=CDS/DB_XREF=gi:3766260/UG=Hs.247763 Human DNA sequence from clone 1032F13 | -3.69       |
| 210721_s_at     | p21(CDKN1A)-activated kinase 7                                       | -3.63       |
| 210327_s_at     | Alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase) | -3.57       |
| 206265_s_at     | Glycosylphosphatidylinositol specific phospholipase D1                | -3.54       |
| 205847_at       | Protease, serine, 22                                                | -3.52       |
| 202901_s_at     | Cathepsin S                                                         | -3.42       |
| 204681_s_at     | Rap guanine nucleotide exchange factor (GEF) 5                       | -3.35       |
| 222227_at       | Zinc finger protein 23                                               | -3.35       |
| 207465_at       | PRO0628 protein                                                     | -3.34       |

STAT3; and upregulation of these genes by Ro25-7386 was confirmed as demonstrated in Fig. 6. The alterations in fold induction of protein levels of certain genes were confirmed by western blot analysis; thus upregulation of BAX, CDH1,
Table VIII. Genes upregulated and downregulated by LG100268 in MDA-MB-231 cells.

A, Genes upregulated by LG100268 in MDA-MB-231

| Probe set | Gene Description | Fold change |
|-----------|------------------|-------------|
| 219948_s_at | Hypothetical protein FLJ21934 | 88.95 |
| 207750_at | gb:NM_018510.1/DEF=Homo sapiens hypothetical protein PRO1866 (PRO1866), mRNA./FEA=mRNA/GEN=PRO1866/PROD=hypothetical protein PRO1866/DB_XREF=gi:8924091/UG=Hs.283031 hypothetial protein PRO1866/PL=gb:AF119858.1 gb:NM_018510.1 | 26.42 |
| 209672_s_at | Hypothetical protein FLJ20323 | 14.63 |
| 215514_at | Consensus includes gb:AL080072.1/DEF=Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616)/FEA=mRNA/DB_XREF=gi:5262482/UG=Hs.21195 Homo sapiens mRNA; cDNA DKFZp564M0616 (from clone DKFZp564M0616) | 9.11 |
| 215309_at | Transcribed locus, weakly similar to XP_092995.4 zinc finger protein 21 (KOX 14) [Homo sapiens] | 8.12 |
| 214774_s_at | Trinucleotide repeat containing 9 | 7.58 |
| 203603_at | Zinc finger homeobox 1b | 5.77 |
| 205386_s_at | Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) | 5.20 |
| 205419_at | Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor) | 4.18 |
| 216978_s_at | Consensus includes gb:U50277.1/DEF=Human breast cancer suppressor element Ishmael Upper CPI1 mRNA, partial cds./FEA=mRNA/PROD=suppressor element Ishmael Upper CP1/DB_XREF=gi:1224126/UG=Hs.121485 Human breast cancer suppressor element Ishmael Upper CP1 mRNA, partial cds. | 3.93 |
| 220931_at | Hypothetical protein MGC5590 | 3.81 |
| 219995_s_at | Hypothetical protein FLJ13841 | 3.77 |
| 208076_at | Histone 1, H4d | 3.6 |
| 214255_at | ATPase, Class V, type 10A | 3.55 |
| 207987_at | Gonadotropin-releasing hormone 1 (luteinizing-releasing hormone) | 3.52 |
| 205651_s_at | Rap guanine nucleotide exchange factor (GEF) 4 | 3.46 |
| 220401_at | Hypothetical protein FLJ21369 | 3.39 |
| 207241_at | Chromosome 4 open reading frame 6 | 3.35 |
| 215623_s_at | SMC4 structural maintenance of chromosomes 4-like 1 (yeast) | 3.17 |
| 216119_s_at | Chromosome 20 open reading frame 28 | 3.13 |
| 217194_at | Consensus includes gb:AB007970.1/DEF=Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501./FEA=mRNA/DB_XREF=gi:3413945/UG=Hs.223020 Homo sapiens mRNA, chromosome 1 specific transcript KIAA0501 | 3.10 |
| 206381_at | Sodium channel, voltage-gated, type II, α 2 | 3.09 |
| 212182_at | Nudix (nucleoside diphosphate linked moiety X)-type motif 4 | 2.98 |
| 215112_s_at | MCF2 cell line derived transforming sequence-like 2 | 2.94 |
| 213747_at | Consensus includes gb:AA047234/FEA=EST/DB_XREF=gi:1525134/DB_XREF=est:zf50b09.s1/CLONE=IMAGE:380345/UG=Hs.223014 antizyme inhibitor | 2.84 |
| 221683_s_at | Centrosome protein cep290 | 2.80 |
| 211611_s_at | Tenascin XB///tenascin XB///cAMP responsive element binding protein-like 1///cAMP responsive element binding protein-like 1 | 2.74 |
| 205421_at | Solute carrier family 22 (extraneuronal monoamine transporter), member 3 | 2.66 |
| 213764_s_at | Microfibrillar associated protein 5 | 2.62 |
| 217505_at | Hypothetical protein MGC22679 | 2.61 |
| 222320_at | Consensus includes gb:AW970584/FEA=EST/DB_XREF=gi:8160429/DB_XREF=est:EST382665/UG=Hs.291033 ESTs | 2.61 |
| 216466_at | Neuron navigator 3 | 2.59 |
| AFFX-r2- | E. coli/GEN=biob/DX_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides | 2.55 |
| Ec-bio | 2393-2682 of gb:J04423.1/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase | 2.66 |
| B-M_at | (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), biotin synthetase, and dethiobiotin synthetase | 2.62 |
| 216775_at | Ubiquitin specific protease 53 | 2.61 |
| 206201_s_at | Mesenchyme homeo box 2 (growth arrest-specific homeo box) | 2.54 |
| AFFX- | E. coli/GEN=biob/DX_XREF=gb:J04423.1/NOTE=SIF corresponding to nucleotides | 2.54 |
| BioDn-5_at | 4980-5256 of gb:J04423.1, not 100% identical/DEF=E.coli 7,8-diamino-pelargonic acid (bioA), | 2.48 |
### Table VIII. Continued.

| Probe set     | Gene                                                                 | Fold change |
|---------------|----------------------------------------------------------------------|-------------|
| 216894_x_at   | biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro | 2.46        |
| 208019_at     | Zinc finger protein 157 (HZF22)                                      | 2.45        |
| 215803_at     | Hypothetical protein FLJ10178                                       | 2.44        |
| 222320_at     | CDNA: FLJ23194 fis, clone REC00490                                  | 2.44        |

**B. Genes downregulated by LG100268**

| Probe set     | Gene                                                                 | Fold change |
|---------------|----------------------------------------------------------------------|-------------|
| 217237_at     | Zinc finger protein 423                                             | -78.6       |
| 215014_at     | Consensus includes gb:AL512727.1/DEF=Homo sapiens mRNA; cDNA DKFZp547P042 (from clone DKFZp547P042) | -5.58       |
| 213753_x_at   | Eukaryotic translation initiation factor 5A                         | -7.65       |
| 212382_at     | Transcription factor 4                                              | -4.82       |
| AFFX-         | H. sapiens/GEN=18S rRNA/DB_XREF=gb:M10098.1/NOTE=SIF corresponding to HUMRGE/M10098_5_at | -4.31       |
| 211712_s_at   | Annexin A9///annexin A9                                             | -5.49       |
| 209227_at     | Tumor suppressor candidate 3                                        | -5.11       |
| 216917_s_at   | Synaptonemal complex protein 1                                      | -3.97       |
| AFFX-         | H. sapiens/GEN=18S rRNA/DB_XREF=gb:M10098.1/NOTE=SIF corresponding to HUMRGE/M10098_M_at | -3.38       |
| 204422_s_at   | Fibroblast growth factor 2 (basic)                                  | -4.11       |
| 209657_s_at   | Heat shock transcription factor 2                                   | -3.96       |
| 221009_s_at   | Angiopoietin-like 4                                                 | -3.90       |
| 205612_at     | Multimerin 1                                                        | -3.79       |
| 207613_s_at   | Calcium/calmodulin-dependent protein kinase (CaM kinase) II α        | -3.55       |
| 37232_at      | KIAA0586                                                           | -3.38       |
| AFFX-         | Signal recognition particle 68 kDa                                  | -3.37       |
| HUMRGE/M10098_3_at | Consensus includes gb:AA292281/FEA=EST/DB_XREF=gi:1940261/DB_XREF =est:zt51b03.s1/CLONE=IMAGE:725837/UG=Hs.181307 H3 histone, family 3A | -3.25       |
| 208453_s_at   | X-prolyl aminopeptidase (aminopeptidase P) 1, soluble               | -3.20       |
| 207485_s_at   | Butyrophilin, subfamily 3, member A1                                | -3.18       |
| 211032_at     | COBL-like 1//COBL-like 1                                             | -3.11       |
| 226019_at     | Chromodomain helicase DNA binding protein 7                         | -3.04       |
| 209318_s_at   | Pleiomorphic adenoma gene-like 1                                    | -3.00       |
| 201547_at     | Jumonji, AT rich interactive domain 1B (RBP2-like)                  | -2.99       |
| 206996_s_at   | Calcium channel, voltage-dependent, β1 subunit                      | -2.98       |
| 220114_s_at   | Stabilin 2                                                          | -2.95       |
| 216799_at     | Hypothetical gene supported by BC013370; BC034583                   | -2.93       |
| 203555_at     | Protein tyrosine phosphatase, non-receptor type 18 (brain-derived)  | -2.92       |
| 13267_at      | KIAA1117                                                           | -2.91       |
interleukin α6 and the downregulation of CDC42 is shown in Fig. 7.

Thorough investigation of the notable genes-CDH1, FOXO3A, BAX (HMEC-Ro25-7386), insulin-like growth factor binding protein 7 and growth differentiation factor 8 (T47D-Ro25-7386) and cathepsin S, TGFβ2, basigin, MCL-1 and BCL2L1 (MCF-7-Ro25-7386), may aid in the clarification of how RXRα agonists function to inhibit breast cell growth. Such notable genes are implicated in breast cancer management and are important for the treatment of breast cancer.

Table VIII. Continued.

| Probe set    | Gene                                      | Fold change |
|--------------|-------------------------------------------|-------------|
| 201122_x_at  | Eukaryotic translation initiation factor 5A | -2.89       |
| 213495_s_at  | gb:AW166067/DB_XREF=xf44g10.x1/CLONE=IMAGE:2620962/FEA=EST/CNT=75/TID=Hs.98614.2/TIER=Stack/STK=51/UG=Hs.98614/LL=6238/UG_GENE=RBBP1/UG_TITLE=ribosome binding protein 1 (dog 180kD homolog) | -2.89       |
| 220301_at    | Chromosome 18 open reading frame 14       | -2.88       |
| 214837_at    | Albumin                                   | -2.85       |
| 209700_s_at  | Phosphodiesterase 4D interacting protein (myomegalin) | -2.84       |
| 216805_at    | Transcribed locus, moderately similar to XP_375099.1 hypothetical protein LOC283585 [Homo sapiens] | -2.84       |
| 221671_x_at  | Immunoglobulin κ constant                 | -2.79       |
| 214001_x_at  | gb:AW302047/DB_XREF=xf52f08.x1/CLONE=IMAGE:2763783/FEA=EST/CNT=24/TID=Hs.76230.2/TIER=Stack/STK=20/UG=Hs.76230/LL=6204/UG_GENE=RPS10/UG_TITLE=ribosomal protein S10 | -2.72       |
| 210047_at    | Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2 | -2.69       |
| 208367_s_at  | Cytochrome P450, family 3, subfamily A, polypeptide 4 | -2.66       |
| 219252_s_at  | Family with sequence similarity 51, member A1 | -2.65       |
| 205827_at    | Cholecystokinin                           | -2.63       |

Figure 5. Expression levels of RXRα in normal and malignant breast cells. Whole cell lysates from normal (HMECs) and malignant breast cells (MCF-7, T47D, MDA-MB-231 and MDA-MB-435) were analyzed by western blotting with anti-RXRα. The data presented are representative of three independent experiments that gave similar results.

Figure 6. mRNA expression levels of RXRα-regulated genes in HMECs (measurement by RT-qPCR). HMECs were treated with Ro25-7386 (1 µM) for 12 h. Subsequent to treatment, total RNA samples were extracted and RNA samples were subject to RT-qPCR analysis. Data are presented as the mean of three independent experiments (error bars denote the standard deviation; *P<0.05, **P<0.01 and ***P<0.001 vs. control). RT-qPCR, reverse transcription-quantitative polymerase chain reaction; DMSO, dimethyl sulfoxide.

Figure 7. Protein levels of RXRα-regulated genes in HMECs measured by western blot analysis. HMECs were treated with Ro25-7386 (1 µM) for 24 h. Subsequent to treatment, whole cell lysates were analyzed with anti-BAX, anti-E-cadherin, anti-interleukin α6 and anti-CDC42 antibodies. The data presented are representative of three independent experiments that gave similar results.
The current study may aid in the elucidation of novel preventive/therapeutic targets for breast cancer, and may contribute to the development of novel molecules, which may be able to inhibit breast cancer development.

Discussion

In order to investigate the molecular mechanism by which retinoids suppress breast cancer development, the current study focused upon RXR-specific ligands (rexinoids). These have been reported to suppress breast cancer development with minimal toxicity compared with RAR-specific ligands (21), and it was the RXRα isofrom that was specifically focused upon in the present study that serves an important role in tumor suppression.

The human RXRα gene spans over 40 kilobases in size and consists of a minimum of 10 exons separated by introns ranging in size from 700 base pairs (intron 3) to >7.8 kb (intron 4) (26). It was observed that all of the cell lines examined expressed RXRα. Notably, ER-negative breast cancer cells, which do not respond to retinoid treatment, such as MDA-MB-231 and MDA-MB-435 also expressed RXRα. This suggests that RXRα is non-functional, losing DNA binding activity or failing to recruit essential co-activators required for the activation of the gene in ER-negative cells. Different and inappropriate sub-localization of the receptor may also explain the unresponsiveness of the cells to retinoid treatment.

LGD1069, LG100268 and Ro25-7386 were observed to suppress the growth of breast cells, including the normal HMECs and ER-positive breast cancer cells (MCF-7 and T47D). LGD1069 was observed to induce a mild inhibition of MDA-MB-231 cell growth at a dose of 10 μM. LG100268 did not affect the cell growth as compared with LGD1069 in all four breast cancer cell lines suggesting its weaker activity. This result indicates that LGD1069 may possess the ability to inhibit the growth of ER-negative breast cancer.

The genes of interest were selected by referring to the PathArt program, which demonstrated the association between genes of several signaling pathways (data not shown). The alterations in gene expression were then analyzed using the Affymetrix microarray (human genome U133A 2.0) to determine which genes are associated with the inhibition of cell growth induced by the rexinoids. Among them, several genes were identified that are involved in cell death, cell growth/maintenance, signal transduction and response to stimuli, including E-cadherin, CtBP1, integrin β4, integrin α6, PAX, BAX, FOXO3A, STAT3, collagen type VI α3 and CDC42. It was additionally confirmed that Ro25-7386 upregulates the mRNA expression levels of FOXO3A, E-cadherin, BAX, PAX, STAT3, integrin α6 and integrin β4. In addition, Ro25-7386 was observed to increase the levels of BAX, E-cadherin and integrin α6 but reduce the level of CDC42. These results suggest that RXRα may have a role in the prevention and treatment of breast cancer development.

Further investigation regarding the functions of selected genes may aid in the elucidation of novel preventive/therapeutic targets for breast cancer, and may additionally contribute to the development of novel molecules, which may inhibit breast cancer progression.

Acknowledgements

The current study was supported by the Department of Defense Breast Cancer Research Program Grants (grant no. W81XWH04-1-0505). The present study was also supported in part by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (grant no. NRF-2012R1A1A3004797) and in part from the Traditional Korean Medicine Research and Development Project, Ministry of Health & Welfare, Republic of Korea (grant no. BI20014).

References

1. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
2. DeSantis C, Ma J, Bryan L and Jemal A: Breast cancer statistics, 2013. CA Cancer J Clin 64: 52-62, 2014.
3. Abu J, Batuwangala M, Herbert K and Symonds P: Retinoic acid and retinoid receptors: potential chemopreventive and therapeutic role in cervical cancer. Lancet Oncol 6: 712-720, 2005.
4. De Luca LM, Darwiche N, Celli G, Kosa K, Jones C, Ross S and Chen LC: Vitamin A in epithelial differentiation and skin carcinogenesis. Nutr Rev 52: 545-582, 1994.
5. Gudas LJ, Sporn MB and Roberts AB: Cellular biology and biochemistry of the retinoids. In: The Retinoids: Biology, Chemistry and Medicine. Sporn MB, Roberts AB and Goodman DS (eds), Raven Press, Ltd. New York, pp.443-520, 1994.
6. Niles RM: Recent advances in the use of vitamin A (retinoids) in the prevention and treatment of cancer. Nutrition 16: 1084-1089, 2000.
7. Simeone AM and Tari AM: How retinoids regulate breast cancer cell proliferation and apoptosis. Cell Mol Life Sci 61: 1475-1484, 2004.
8. Love JM and Gudas LJ: Vitamin A, differentiation and cancer. Curr Opin Cell Biol 6: 825-831, 1994.
9. Vivat-Hannah V, You D, Rizzo C, Daris JP, Lapointe P, Zusi FC, Marinier A, Lorenzi MV and Gottardis MM: Synergistic cytotoxicity exhibited by combination treatment of selective retinoid ligands with taxol (Paclitaxel). Cancer Res 61: 8703-8711, 2001.
10. Altucci L, Rossin A, Raffelsberger W, Reitmair A, Chomienne C and Gronemeyer H: Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. Nat Med 7: 680-686, 2001.
11. Chambon P: A decade of molecular biology of retinoic acid receptors. FASEB J 10: 940-954, 1996.
12. Giguère V: Retinoic acid receptors and cellular retinoid binding proteins: Complex interplay in retinoid signaling. Endocr Rev 15: 61-79, 1994.
13. Zusi FC, Lorenzi MV and Vivat-Hannah V: Selective retinoids and retinoids in cancer therapy and chemoprevention. Drug Discov Today 7: 1165-1174, 2002.
14. Thacher SM, Vasudevan J and Chandraratna RA: Therapeutic applications for ligands of retinoid receptors. Curr Pharm Des 6: 25-58, 2000.
15. Nagpal S and Chandraratna RA: Recent developments in receptor-selective retinoids. Curr Pharm Des 6: 919-931, 2000.
16. Kong G, Kim HT, Wu K, et al: The retinoid X receptor-selective retinoid, LGD1069, down-regulates cyclooxygenase-2 expression in human breast cells through transcription factor crosstalk: implications for molecular-based chemoprevention. Cancer Res 65: 3462-3469, 2005.
17. Nagy L, Thomazy VA, Heyman RA and Davies PJ: Retinoid-induced apoptosis in normal and neoplastic tissues. Cell Death Differ 5: 11-19, 1998.
18. Lippman SM and Lotan R: Advances in the development of retinoids as chemopreventive agents. J Nutr 130 (Suppl 2): 479S-482S, 2000.
19. Gottardis MM, Bischoff ED, Shirley MA, Wagoner MA, Lamph WW and Heyman RA: Chemoprevention of mammary carcinoma by LGD1069 (Targetin): an RXR-selective ligand. Cancer Res 56: 5566-5570, 1996.
20. Wu K, Zhang Y, Xu XC, et al: The retinoid X receptor-selective retinoid, LGD1069, prevents the development of estrogen receptor-negative mammary tumors in transgenic mice. Cancer Res 62: 6376-6380, 2002.

21. Wu K, Kim HT, Rodriguez JL, et al: Suppression of mammary tumorigenesis in transgenic mice by the RXR-selective retinoid, LGD1069. Cancer Epidemiol Biomarkers Prev 11: 467-474, 2002.

22. Crowe DL and Chandraratna RA: A retinoid X receptor (RXR)-selective retinoid reveals that RXR-alpha is potentially a therapeutic target in breast cancer cell lines and that it potentiates antiproliferative and apoptotic responses to peroxisome proliferator-activated receptor ligands. Breast Cancer Res 6: R546-R555, 2004.

23. Farol LT and Hymes KB: Bexarotene: a clinical review. Expert Rev Anticancer Ther 4: 180-188, 2004.

24. Rigas JR and Dragnev KH: Emerging role of rexinoids in non-small cell lung cancer: focus on bexarotene. Oncologist 10: 22-33, 2005.

25. Ma Y, Koza-Taylor PH, DiMattia DA, Hames L, Fu H, Dragnev KH, Turi T, Beebe JS, Freemantle SJ and Dmitrovsky E: Microarray analysis uncovers retinoid targets in human bronchial epithelial cells. Oncogene 22: 4924-4932, 2003.

26. Li G, Walch E, Yang X, Lippman SM and Clifford JL: Cloning and characterization of the human retinoid X receptor alpha gene: conservation of structure with the mouse homolog. Biochem Biophys Res Commun 69: 54-57, 2000.