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BREAST CANCER DEVELOPMENT

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Molecular mechanism by which retinoids prevent breast cancer development

**Identification of biomarkers regulated by the retinoids (Ro25-7386,
LGD1069 and LG100268) in human breast cells
using Affymetrix Microarray**

Final report, June 30, 2007

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INTRODUCTION

We investigated whether rexinoids suppress the growth of breast cells, and how they act to inhibit breast cancer development. First, we measured the growth suppressive activity of rexinoids (RO 25-7386, LGD1069 and LG100268) on the growth of breast cells; normal breast cells (HMEC), ER-positive breast cancer cells (MCF-7 and T47D) and ER-negative breast cancer cells (MDA-MB-231 and MDA-MB-435). Second, we identified the genes induced by rexinoids that could be related to the cell growth inhibition. By MTS assay, we found that all three compounds (Ro25-7386, LGD1069 and LG100268) inhibited significantly the growth of normal breast cells, HMEC, at 10 uM suggesting the chemopreventive property of the rexinoids. The growth of MCF-7 cells was inhibited by Ro25-7386. T47D breast cancer cells were found to be growth suppressive by Ro25-7386 and LGD1069; they strongly suppressed the cell growth by dose-dependent manner. Surprisingly, LGD1069 also induced a mild inhibition (20 % growth inhibition) of MDA-MB-231 at 10 uM suggesting that LGD1069 can inhibit the ER-negative breast cancer cell growth in vitro. Later, we performed the Affymetrix microarray to identify the genes induced by rexinoids (Ro25-7386, LGD1069 and LG100268). We found several interesting genes induced by the rexinoids. [\(Please see APPENDICES for the journal article which will be submitted shortly\).](#)

BODY and RESULTS

(Please see APPENDICES for the journal article which will be submitted shortly).

To start the grant research, we planned to follow the approved statement of work using the noticed breast cell lines (HMEC, MCF-7, T47D, MDA-MB-231 and MDA-MB-435), using RXR alpha-bound compound, Ro25-7386. We previously focused on LGD1069 and LG100268 compounds (rexinoids) which randomly bind to RXR isoforms and planed to work on LGD1069-target gene IGFBP-6 to investigate the role of this gene in breast cancer suppression induced by LGD1069 (statement of work).

However, from the first period of this study, we used another specific rexinoid, Ro25-7386 which exclusively bind RXR α isoform. Then, we performed newly Affymetrix microarray data for Ro25-7386, and even LGD1069 and LG100268 to find interesting target genes in normal breast cells and breast cancer cells. Since,

1) We could not obtain the compounds, LGD1069 and LG100268 from the Ligand Pharmaceuticals for the first year.

2) IGF binding protein-6 (IGFBP-6) was reported to be inducible by RA (all-*trans*-retinoic acid) in the retinoid resistant cells, BEAS-2B-R1 human bronchial epithelial cells (Ma et al., 2003).

3) RXR α seems to play a critical role in tumor suppression.

4) We submitted the total RNA samples to the microarray after 12 h time point induction; this time point was selected for study since retinoid treatment would likely regulate the expression of genes earlier than 24 h (Li et al., 2000).

First, we measured the growth suppressive activity of each of rexinoids (Ro25-7386, LGD1069 and LG100268) on the growth of breast cells; normal breast cells (HMEC), ER-positive breast cancer cells (MCF-7 and T47D) and ER-negative breast cancer cells (MDA-MB-231 and MDA-MB-435).

By MTS assay, we found that all three compounds (Ro25-7386, LGD1069 and LG100268) inhibited significantly the growth of normal breast cells, HMEC, at 10 μ M

suggesting the chemopreventive property of the rexinoids. The growth of MCF-7 cells was inhibited by Ro25-7386. T47D breast cancer cells were found to be growth suppressive by Ro25-7386 and LGD1069; they strongly suppressed the cell growth by dose-dependent manner. Surprisingly, LGD1069 also induced a mild inhibition (20 % growth inhibition) of MDA-MB-231 at 10 uM suggesting that LGD1069 can inhibit the ER-negative breast cancer cell growth in vitro.

Later, we performed the Affymetrix microarray to identify the target genes induced by rexinoids (Ro25-7386, LGD1069 and LG100268) in each cell line which demonstrated the growth suppressive activity. We found several interesting genes induced by the rexinoids. We selected our major genes of interest by referring to PathArt program which demonstrate the relationship between genes by several signaling pathways.

In HMEC, we identified 638 genes up-regulated and 347 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold. 22 genes were strongly up-regulated (more than 10 fold), and 5 genes were strongly down-regulated (more than 4 fold) in expression levels by Ro25-7386 (Table 1A and 1B). Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus; i.e., cadherin 1, type1, E-cadherin (CDH1), C-terminal binding protein 1 (CtBP1), integrin beta 4, integrin alpha 6, paxillin (PAX), BCL2-associated X protein (BAX), forkhead box O3A (FOXO3A), signal transducer and activator of transcription 3 (STAT3) (up-regulated genes); collagen type VI alpha 3 and cell division cycle 42 (CDC42) (down-regulated genes). In T47D, we found only 3 up-regulated genes and 5 down-regulated genes with changes in fold induction greater than 2 fold. Among them, we found chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (up-regulated genes); Glutamate receptor, ionotropic kainite 2, colon carcinoma related protein, insulin-like growth factor binding protein 7 (IGFBP-7), growth differentiation factor 8, KIAA0998. In MCF-7, we identified 83 genes up-regulated and 98 genes down-regulated by Ro25-7328 with changes in fold induction greater than 2 fold. Among them, we found several genes which are involved in cell

death, cell growth/maintenance, signal transduction and response to stimulus; i.e., TGF beta2, PKC delta binding protein, Neurophilin 2, Interleukin 6 receptor (up-regulated genes); TGF beta1, Integrin beta 4., Paxillin (PAX), Tumor protein 53 (TP53), C-terminal binding protein 1 (CtBP-1), Mcl-1, Bcl-like1 (down-regulated genes).

In T47D, 16 up-regulated genes and 3 down-regulated genes modulated by LGD1069; 3 up-regulated genes and 5 down-regulated genes were modulated by LGD100268 with changes in fold induction greater than 2 fold. In MDA-MB-231, we identified 333 genes up-regulated and 319 genes down-regulated by LGD1069 with changes in fold induction greater than 2 fold. We also identified 116 genes up-regulated and 431 genes down-regulated by LG100268 with changes in fold induction greater than 2 fold. According to the data, we found several interesting genes induced by LGD1069 and LG100268 in MDA-MB-231 such as several hypothetical proteins, CDC14 cell division cycle 14 homolog A (*S. cerevisiae*), recombination activating gene 2, tumor protein D52, MDM2, ITGA4, ADH1B, NF2 and cathepsin S (for LGD1069), several hypothetical proteins, zinc finger protein 423, cyclin-dependent kinase inhibitor 1C (p57, Kip2), eukaryotic translation initiation factor 5A, transcription factor 4, MDM2, FGF2, GNRH1, and annexin A9 (for LG100268). We will study their functions. We are also performing gene profiling for HMEC to identify the genes regulated by LGD 1069 and LG100268 – this step is still on-going.

KEY RESEARCH ACCOMPLISHMENTS

1. Ro25-7386, LGD1069 and LG100268 strongly suppressed the growth of normal breast cells (HMEC) displaying their potential chemopreventive activity.
2. Ro25-7386 and LGD1069 strongly suppressed the growth of T47D. MDA-MB-231 cells displaying its therapeutic role in ER-positive as well as ER-negative breast cancer.
3. Gene profiling for RXR α -target genes induced by Ro25-7386 in normal HMEC.
4. Gene profiling for RXR α -target genes induced by Ro25-7386 in T47D.
5. Gene profiling for RXR α -target genes induced by Ro25-7386 in MCF-7.
6. Gene profiling for LGD1069 and LG100268 in normal HMEC.
7. Gene profiling for LGD1069 and LG100268 in MCF-7.
8. Gene profiling for LGD1069 and LG100268 in MDA-MB-231.

REPORTABLE OUTCOMES

1. Some part of this work was presented in **2005 Era of Hope meeting (poster presentation- #poster number; p36-13 #abstract – proceedings ; pp 253)**
2. Some part of this work was presented in **2006 AACR International Conference on Frontiers in Cancer Prevention Research (poster presentation- #poster number; #126)**
3. Some part of this work was presented in **2007 AACR annual meeting (poster presentation- #poster number; #4420)**

CONCLUSION

(Please see [APPENDICES](#) for the journal article which will be submitted shortly).

We have studied the molecular mechanism by which retinoids suppress breast cancer development. For that purpose, we tested the growth suppressive activity of the rexinoids (Ro25-7386, LGD1069 and LG100268) in human normal and malignant breast cells. Then, we identified biomarkers regulated by the rexinoids in human normal and malignant breast cells using Affymetrix Microarray.

According to our study, it is demonstrated that LGD1069 might be useful for the prevention and treatment of both ER-positive and ER-negative breast cancer. Moreover, LG100268 which is shown its weaker activity compare to LGD1069 should be more chemopreventive than chemotherapeutic.

This study could help us to find new preventive/therapeutic target for breast cancer, and may contribute to develop novel molecule which could inhibit breast cancer development.

Further, we need to confirm the expression levels of mRNA and protein by real-time RT-PCR and western blot analysis. We also need to study the function of selected genes through the use of the recombinant protein and siRNA transfection. This study could help us to find new preventive/therapeutic target for breast cancer, and may contribute to develop novel molecule which could inhibit breast cancer development.

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APPENDICES

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

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ABSTRACT

In this study, we identified genes modulated by rexinoids (RXR-pan agonist; LGD1069 and LG100268) and RXR- α agonist (Ro25-7386)-regulated genes by

affymetrix microarray in normal and malignant breast cells. Ro25-7386, LGD1069 and LG100268 suppressed strongly the growth of normal HMEC (human mammary epithelial cells) at 10 μ M suggesting the chemopreventive property of the rexinoids. The growth of MCF-7 cells was inhibited by Ro25-7386. T47D breast cancer cells were found to be growth suppressive by Ro25-7386 and LGD1069; they strongly suppressed the cell growth by dose-dependent manner. Surprisingly, LGD1069 also induced a mild inhibition (20 % growth inhibition) of MDA-MB-231 at 10 μ M suggesting that LGD1069 can inhibit the ER-negative breast cancer cell growth in vitro.

Later, to identify genes that are regulated by rexinoids-regulated genes, we treated each cell line (HMEC, T47D, MCF-7, MDA-MB-231) with each rexinoid (Ro25-7386, LGD1069 and LG100268) for 12h using the dose which most strongly suppressed the cell growth (10 μ M). We then examined changes in gene expression by using the affymetrix microarray (human genome U133A 2.0) to further investigate which genes are related to the cell growth inhibition induced by the rexinoids.

In HMEC, we identified 638 genes up-regulated and 347 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold. In T47D, we found only 3 up-regulated genes and 5 down-regulated genes with changes in fold induction greater than 2 fold when treated with Ro25-7386. In MCF-7, we identified 83 genes up-regulated and 98 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold.

In T47D, 16 up-regulated genes and 3 down-regulated genes modulated by LGD100268 with changes in fold induction greater than 2 fold. In MDA-MB-231, 333 up-regulated genes and 319 down-regulated genes modulated by LGD1069; 118 up-regulated genes and 432 down-regulated genes were modulated by LG100268 with changes in fold induction greater than 2 fold. Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus. These rexinoid-regulated genes may be associated with growth suppressive activity of Ro25-7386, LGD1069 or LG100268 in breast cells. Also they can be served as biomarkers and new molecular targets for the prevention and treatment for the breast cancer using rexinoids.

INTRODUCTION

Breast cancer is the most diagnosed cancer in women and the second leading cause of death from cancer in women (1). In 2007, the ACS estimates 178,480 women will be newly diagnosed with breast cancer, and 40,460 women will be died from this disease (2). Ultimate purpose of scientist and clinicians to conquer this disease is to prevent the incidence, detect early and treat breast cancer with effective therapy resulting in long overall survival minimizing side effects. Therefore, the purpose of this study is to find the way to prevent and treat breast cancer identifying the genes related to the cell growth inhibition which are induced by Retinoid X receptor (RXR)-selective retinoids (rexinoids).

Retinoids regulate a variety of biological functions such as embryogenesis, growth, differentiation, vision and reproduction (3-6). Retinoids also contain anti-proliferative properties, demonstrating their chemopreventive and therapeutic role against cancer (7). Moreover, retinoids is known to inhibit normal- or tumor-cell growth through the regulation of differentiation and/or apoptosis (8-11).

The retinoids exert their effects in target cells interacting with RARs and RXRs,. Each of these includes three subtypes, designated α , β and γ , which are encoded by distinct genes. The $RAR\alpha$, $RAR\beta$, and $RAR\gamma$ genes have been localized to chromosomes 17q21, 3p24 and 12q13, respectively. The $RXR\alpha$, $RXR\beta$, and $RXR\gamma$ genes have been mapped to chromosomes 9q34.3, 6p21.3 and 1q22-23, respectively (12). The RAR bind both ATRA and 9-*cis* RA while RXR bind only 9-*cis* RA. RAR can form

heterodimers with RXR. RXRs are well-known to heterodimerize with several steroid hormone receptors, such as RAR, TR, VDR, PPAR, LXR, PXR and FXR suggesting its involvement in several signaling pathways. RXR also homodimerize in transfected cells (13).

In addition to naturally occurring retinoids, such as all-*trans* retinoic acid (ATRA), 9-*cis* retinoic acid (9-*cis*-RA) and 13-*cis* retinoic acid (13-*cis*-RA), various synthetic retinoids with different selectivity have been developed and are currently available to treat psoriasis, acne, photoaging, actinic keratosis or cancers such as acute promyelocytic 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 include cheilitis, hypertriglyceridemia and hepatosplenomegaly (15).

Rexinoids are important in controlling apoptosis and can function in a ligand-dependent or ligand-independent manner (16, 17). Importantly, rexinoids are known to suppress both ER-positive and ER-negative mammary tumor development with reduced toxicity compared to RAR-selective retinoids (18-20). Rexinoids are also active in animals in tamoxifen-resistant breast cancer (17, 21) and in ATRA-resistant breast cancer cells (23). Hence, rexinoids seems to be more efficient and promising chemopreventive and therapeutic drugs compare to RAR-selective ligands. Among them, LGD1069 (Bexarotene) was confirmed as safe and well tolerated agent in clinical trials of cutaneous T-cell lymphoma, breast cancer and lung cancer (23-24)

We, therefore, are interested in rexinoid and its cognate receptor, RXR in breast cells studying their regulatory activity in transcription of genes involved in growth suppression. We concentrated our focus on the RXR α isoform which is a potential therapeutic target in breast cancer cells since over-expression of RXR α sensitized breast cancer cells lines to the anti-proliferative effect of RXR-selective ligand (25), moreover, infection of adenoviral RXR α induced nucleoplasmic over-expression of RXR α and resulted in apoptosis under treatment with an RXR ligand in retinoid-resistant MDA-MB-231 (27). Hence, in this study, we tested 1) the growth suppressive activity of RXR α specific ligand, Ro25-7386 in normal human mammary epithelial cells (HMEC) and retinoid-sensitive breast cancer cells (T47D and MCF-7) by MTT assay, 2) then identified genes regulated by RXR α which may be involved in the anti-proliferative activity of Ro25-7386 by Affymetrix microarray. We found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus. Further studying the function of those genes, we may seek to clarify the mechanism by which rexinoids suppress breast cancer development.

Materials and Methods

Ligands and Reagents

RXR α -selective ligand, Ro 25-7386 was generous gifts from kindly provided by Roche Bioscience, Palo Alto, CA). This compound was diluted in DMSO, purchased from Sigma Chemical Co. (St Louis, MO) giving a final concentration of 0.1 %. Monoclonal or polyclonal antibodies (mouse or rabbit) containing E-cadherin, FKHRL1, Integrin α 6, Integrin β 4, Bax, transforming growth factor, beta 2 and cdc42 rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cells and Culture Materials

Human normal mammary epithelial cells (HMEC) were obtained from Clonetics (San Diego, CA). They were obtained as primary cultures derived from healthy women who had undergone reduction mammoplasties. Cells between passages 10 and 11 were used for experiments. Cells were grown and maintained in mammary epithelial basal medium (MEBM) supplemented 13 mg/ml bovine pituitary extract, 0.5 % serum, 5 μ g/ml insulin, 10 ng/ml human recombinant epidermal growth factor (EGF), 0.5 mg/ml hydrocortisone, 50 μ g/ml gentamicin, and 50 μ g/ml amphotericin- β (Clonetics, San Diego, CA). Cells were maintained in a humidified environment at 37 °C with 5 % CO₂ in air.

Four different human breast cancer cells (MCF-7, T47D, MDA-MB-231 and MBA-MB-435 cells) purchased from ATCC (Manassas, VA) were grown and maintained in appropriate growth media; minimal essential medium (MEM) for MCF-7, RPMI 1740 for

T47D, MDA-MB-231 and MBA-MB-435 with phenol red (In Vitrogen™, Life Technologies: Carlsbad, CA) supplemented with 10% heat-inactivated FBS. L-glutamine, penicillin, streptomycin and gentamicin were used at the usual concentrations. For all experiments, breast cancer cells were harvested by trypsinization (0.25% trypsin - 0.02% EDTA), 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[®] AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) was used for the measurement of cell growth rate in breast cancer cells according to the manufactory's protocol. The CellTiter 96[®] AQueous Assay is composed of solutions of a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS). Briefly, HMEC, T-47D and MCF-7 (each cell line-1,000 cells/well), were plated in 96-multi plastic wells in MEBM (see above). The next day, Ro 25-7386, LGD1069 and LG100268 were added in the growth media, and cell culture was pursued for the following days. Each testing day, MTS were added to the cells, and further incubation was pursued for 2 h. MTS is bio-reduced by cells into a formazan product that is soluble in tissue culture medium. The quantity of formazan product as measured by the amount of 490nm absorbance. Each data point was performed in quadruplet and the results were reported as mean absorption (optic density, OD)

Real-time RT-PCR analysis

HMEC and MCF-7 cells were cultured until 80%-90% confluence in MEBM. Total RNA was prepared by using the Qiagen RNeasy mini kit (Qiagen, Maryland). cDNA of the 10 selected genes and an internal reference gene (glyceraldehydes-3-phosphate dehydrogenase-GAPDH) was made from each sample, then quantified using a fluorescence-based real-time detection method [Bio-Rad iCycler (Hercules, CA)]. RT-PCR analysis was performed by the standard methods recommended by the RT-PCR kit supplier (Applied Biosystems, Foster City, CA). Primers sequences used for detection of the level of CREB were described in Table 5. For endogeneous control, human GAPDH labeled with VIC[™] dye – provided by the company was used. The amplification conditions were as follows: 30 s at 95 °C, 3 min at 95 °C, and 30 s at 95 °C and 60 s at 65 °C for 40 cycles, followed by a final extension for 20 min at 72 °C. The ratio between the values obtained provided relative gene expression levels.

Western Blot analysis

HMEC, retinoid-sensitive breast cancer cells (T47D and MCF-7), and retinoid-insensitive breast cancer cell lines (MDA-MB-231 and MDA-MB-435) are cultured in culture medium. Whole cell extracts of each cell line were prepared using 1 X sodium dodecyl sulfate (SDS) Laemmli lysis buffer (125 mM Tris-HCl pH6.8, 1% SDS, 2% β -mercaptoethanol, 0.01%). Total cell lysates with equal amounts of protein (10 μ g) were subjected to 10% SDS-PAGE and subsequently electrotransferred onto a nitrocellulose membrane (Bio-Rad laboratories, Hercules, CA). Membranes were blocked with 5% skim milk in PBST (PBS containing 0.1% Tween20) for 1 h at room temperature then were

incubated overnight with primary antibody in PBST containing 2.5% BSA (1:1000 dilution). After 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 Corp., NY) in PBST and then visualized by using the ECL system (Amersham Biosciences, Arlington Heights, IL). Protein expression was normalized using beta-actin expression.

Results

Expression of RXR α in breast cells

In Figure 1, the structure of LGD1069 and LG100268 are displayed. We first determined the expression level of RXR α in 1 normal breast cells (HMEC), two retinoid-sensitive breast cancer cells (MCF-7 and T47D) and two retinoid-insensitive breast cancer cells (MDA-MB-231 and MDA-MB-435). We found that all breast cell lines express RXR α but with different intensity. MCF-7 and T47D expressed higher amount of RXR α (Figure 2). Interestingly, ER-negative breast cancer cell lines which do not respond to retinoid treatment such as MDA-MB-231 and MDA-MB-435 also expressed RXR α .

Anti-proliferative activity of RXR α -specific ligand

Secondly, we tested the anti-proliferative effect of RXR α specific compound, Ro25-7386 in HMEC. By MTS assay, we found that Ro25-7386 suppressed HMEC normal breast cell growth by dose-dependent manner. We also found that Ro25-7386 suppressed T47D cell growth at 1 μ M. Ro25-7386 induced mild suppression of the cell growth in MCF-7 at 1 μ M (Figure 3). These results indicate that RXR α agonist, Ro25-7386 suppresses the growth of HMEC and retinoid-sensitive breast cancer cells, T47D and MCF-7. Hence, RXR α seems to play an important role for growth suppression induced by retinoid in breast cells. Moreover, the amount of RXR α receptor may not be related to the inhibition of breast cell growth induced by Ro25-7386. These results indicate that RXR α agonist, Ro25-7386 suppressed the growth of HMEC and retinoid-

sensitive breast cancer cells, T47D and MCF-7. Hence, RXR α seems to play an important role “at least in part” for growth suppression induced by rexinoid in breast cells. Moreover, the amount of RXR α receptor may not be related to the inhibition of breast cell growth induced by Ro25-7386.

Anti-proliferative activity of RXR α -specific ligand

Third, we tested the anti-proliferative effect of LGD1069 and LG100268 in HMEC, retinoid-sensitive breast cancer cells (T47D and MCF-7), and retinoid-insensitive breast cancer cells (MDA-MB-231 and MDA-MB-435). By MTS assay, we found that Ro25-7386 suppressed HMEC normal breast cell growth by dose-dependent manner. We also found that Ro25-7386 suppressed T47D cell growth at 1 μ M while Ro25-7386 suppressed T47D cell growth at 1 μ M. Ro25-7386 induced mild suppression of the cell growth in MCF-7 at 1 μ M (Figure 3).

These results indicate that RXR α agonist, Ro25-7386 suppresses the growth of HMEC and retinoid-sensitive breast cancer cells, T47D and MCF-7. Hence, RXR α seems to play an important role for growth suppression induced by rexinoid in breast cells. Moreover, the amount of RXR α receptor may not be related to the inhibition of breast cell growth induced by Ro25-7386. These results indicate that RXR α agonist, Ro25-7386 suppressed the growth of HMEC and retinoid-sensitive breast cancer cells, T47D and MCF-7. Hence, RXR α seems to play an important role “at least in part” for growth suppression induced by rexinoid in breast cells. Moreover, the amount of RXR α receptor may not be related to the inhibition of breast cell growth induced by Ro25-7386.

Anti-proliferative activity of LGD1069 and LG100268

Third, we tested the anti-proliferative effect of LGD1069 and LG100268 in HMEC, retinoid-sensitive breast cancer cells (T47D and MCF-7), and retinoid-insensitive breast cancer cells (MDA-MB-231 and MDA-MB-435). After 8 days of treatment, we found that LGD1069 suppressed the growth of HMEC by dose-dependent manner; by MTS assay, LG100268 suppressed strongly the growth of HMEC at 10 μ M (Figure 4). On the other hand, when cells were submitted to the co-treatment of LGD1069 and LG100268, we could not observe the synergistic effect of the ligands, LGD1069 and LG100268.

Both LGD1069 and LG100268 did not affect the cell growth rate of MCF-7 even at the strongest dose, 10 μ M. However, T47D displayed its sensitivity to LGD1069 and LG100268. The rexinoids strongly suppressed the cell growth of T47D by dose-dependent manner after 10 days of treatment (Figure 5). Especially, it seems that LGD1069 has stronger activity compare to LG100268 for the cell growth of T47D.

Rexinoid, especially LGD1069 induced a mild inhibition (20 % inhibition) of the cell growth of MDA-MB-231 at the dose of 10 μ M (Figure 6). This result indicates that LGD1069 can inhibit the growth of ER-negative breast cancer with therapeutic potency.

By MTS assay, we found that both LGD1069 and LG100268 inhibited significantly normal HMEC cell growth at 10 μ M. We also found that LGD1069 strongly suppressed the growth of T47D (ER-positive) by dose-dependent manner. LGD1069 also

induced a mild inhibition of MDA-MB-231 (ER-negative) at 10 μ M. MCF-7 and MDA-MB-435 did not have growth suppression by LGD1069 at 10 μ M. LG100268 did affect little the cell growth in all 4 breast cancer cell lines suggesting its weak activity compare to LGD1069 (Figure 6).

We also found that LGD1069 strongly suppressed the growth of T47D (ER-positive) by dose-dependent manner. LGD1069 also induced a mild inhibition of MDA-MB-231 (ER-negative) at 10 μ M.

Determine RXR α target genes of normal and malignant breast cells by Affymetrix microarray

For the next step of the study, we identified genes regulated by RXR α -specific ligand, Ro25-7386 in normal (HMEC) and malignant (T47D and MCF-7) breast cells. Gene expression profiles were established by using Affymetrix microarray (human genome U133A 2.0). In Figure 7, the comparison between cell growth suppressive activity and Affymetrix Microarray data was shown. For that purpose, we treated breast cells with Ro25-7386 using the concentration which most strongly suppressed the cell growth (10 μ M), and total RNA sample was harvested after 12h; this time point was selected for study since retinoid treatment would likely regulate the expression of genes earlier than 24h (28). We then examined changes in gene expression by using the microarray to investigate which genes are related to cell growth inhibition induced by the RXR α agonist. In HMEC, we identified 638 genes up-regulated and 347 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold. 22 genes were

strongly up-regulated (more than 10 fold), and 5 genes were strongly down-regulated (more than 4 fold) in expression levels by Ro25-7386 (Table 1A and 1B).

In HMEC, we identified 638 genes up-regulated and 347 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold. 22 genes were strongly up-regulated (more than 10 fold), and 5 genes were strongly down-regulated (more than 4 fold) in expression levels.

Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus; i.e., cadherin 1, type1, E-cadherin (CDH1), C-terminal binding protein 1 (CtBP1), integrin beta 4, integrin alpha 6, paxillin (PAX), BCL2-associated X protein (BAX), forkhead box O3A (FOXO3A), signal transducer and activator of transcription 3 (STAT3) (up-regulated genes); collagen type VI alpha 3 and cell division cycle 42 (CDC42) (down-regulated genes). In T47D, we found only 3 up-regulated genes and 5 down-regulated genes with changes in fold induction greater than 2 fold. Among them, we found chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (up-regulated genes); Glutamate receptor, ionotropic kainite 2, colon carcinoma related protein, insulin-like growth factor binding protein 7 (IGFBP-7), growth differentiation factor 8, KIAA0998 (Table 2A and 2B). In MCF-7, we identified 83 genes up-regulated and 98 genes down-regulated by Ro25-7328 with changes in fold induction greater than 2 fold (Table 3A and 3B). Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus; i.e., TGF beta2, PKC delta binding protein,

Neurophilin 2, Interleukin 6 receptor (up-regulated genes); TGF beta1, Integrin beta 4., Paxillin (PAX), Tumor protein 53 (TP53), C-terminal binding protein 1 (CtBP-1), Mcl-1, Bcl-like1 (down-regulated genes).

In T47D, 16 up-regulated genes and 3 down-regulated genes modulated by LGD1069 (Table 4A and 4B); 3 up-regulated genes and 5 down-regulated genes were modulated by LGD100268 with changes in fold induction greater than 2 fold (Table 5A and 5B). In MDA-MB-231, 335 up-regulated genes and 320 down-regulated genes modulated by LGD1069 (Table 6A and 6B); 118 up-regulated genes and 432 down-regulated genes were modulated by LGD100268 with changes in fold induction greater than 2 fold (Table 7A and 7B). According to the data, we found several interesting genes induced by LGD1069 and LG100268 in MDA-MB-231 such as several hypothetical proteins, CDC14 cell division cycle 14 homolog A (*S. cerevisiae*), recombination activating gene 2, tumor protein D52, MDM2, ITGA4, ADh1B, NF2 and cathepsin S (for LGD1069), several hypothetical proteins, zinc finger protein 423, cyclin-dependent kinase inhibitor 1C (p57, Kip2), eukaryotic translation initiation factor 5A, transcription factor 4, MDM2, FGF2, GNRH1, and annexin A9 (for LG100268). We will study their functions.

Confirmation of the changes of modulation of RXR α target genes of HMEC by real-time RT-PCR and western blot analysis

The induction of a total of 7 genes by rexinoid (mRNA levels) was confirmed by real-time RT-PCR assays. They include integrin beta 4, integrin alpha 6, E-cadherin

(CDH1), PAX, BAX, FOXO3A and STAT3; up-regulation of those genes by Ro25-7386 was confirmed (Figure 8). The primers for forward and reverse directions were described in Table 8. The changes in fold induction of protein levels of some genes were confirmed by western blot analysis; up-regulation of BAX, E-cadherin, interleukin alpha 6 and the down-regulation of CDC42 were revealed (Figure 9).

Profound investigation of our interesting genes - CDH1, FOXO3A and BAX (HMEC-Ro25-7386), insulin-like growth factor binding protein 7 (IGFBP-7) and growth differentiation factor 8 (T47D-Ro25-7386) and cathepsin S, TGF β 2, basigin, MCL-1 and BCL2L1 (MCF-7-Ro25-7386), CDH1), C-terminal binding protein 1 (CtBP1) may lead us to clarify how RXR α agonist functions to inhibit breast cell growth. This study could help us to find new preventive/therapeutic target for breast cancer, and may contribute to develop novel molecule which could inhibit breast cancer development.

Discussion

To reach our ultimate goal – study the molecular mechanism by which retinoids suppress breast cancer development, we focused our attention on RXR-specific ligands (rexinoids) which have been reported to suppress breast cancer development with minimal toxicity compare to RAR-specific ligands. We also especially focused our study toward RXR α isoform, which plays an important role in tumor suppression.

Human RXR α gene spans over 40 kilobases in size and consists of at least 10 exons separated by introns ranging in size from 700 base pairs (intron 3) to more than 7.8 kb (intron 4) (28). We observed that all cell lines tested expressed RXR α . Interestingly, ER-negative breast cancer cells which do not respond to retinoid treatment such as MDA-MB-231 and MDA-MB-435 also expressed RXR α . This suggests that RXR α is non-functional losing DNA binding activity or failing to recruit essential co-activators required for the gene activation in retinoid-insensitive cells. Different and inappropriate sub-localization of the receptor may also explain the unresponsiveness of the cells to retinoid.

We found that LGD1069, LG100268 and RXR α agonist, Ro25-7386 suppressed the growth of breast cells - normal HMEC cells and retinoid-sensitive breast cancer cell lines (MCF-7 and T47D). We found that LGD1069, LG100268 and RXR α agonist Ro25-7386 suppressed the growth of breast cells – normal HMEC cells and retinoid-sensitive breast cancer cell line, T47D. MCF-7 breast cancer cell line was shown as growth

suppressive for Ro25-7386 but was not growth suppressive for LGD1069 and LG100268. As expected, Ro25-7386 had no growth suppressive activity on retinoid-insensitive breast cancer cell lines, MDA-MB-231 and MDA-MB-435 suggesting that RXR α existed in those cell lines is non-functional (data not shown). Surprisingly, especially LGD1069 induced a mild inhibition (20 % inhibition) of the cell growth of MDA-MB-231 at the dose of 10 μ M. LG100268 did affect little the cell growth compare to LGD1069 in all 4 breast cancer cell lines suggesting its weaker activity. This result indicates that LGD1069 can inhibit the growth of ER-negative breast cancer with therapeutic potency.

We selected our major genes of interest by referring to PathArt program which demonstrate the relationship between genes by several signaling pathways. We then examined changes in gene expression by using the affymetrix microarray (human genome U133A 2.0) to further investigate which genes are related to the cell growth inhibition induced by the rexinoids.

In HMEC, we identified 638 genes up-regulated and 347 genes down-regulated by Ro25-7386 with changes in fold induction greater than 2 fold. 22 genes were strongly up-regulated (more than 10 fold), and 5 genes were strongly down-regulated (more than 4 fold) in expression levels by Ro25-7386 (Table 1A and 1B).

Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus; i.e., cadherin 1, type1, E-cadherin (CDH1), C-terminal binding protein 1 (CtBP1), integrin beta 4, integrin alpha

6, paxillin (PAX), BCL2-associated X protein (BAX), forkhead box O3A (FOXO3A), signal transducer and activator of transcription 3 (STAT3) (up-regulated genes); collagen type VI alpha 3 and cell division cycle 42 (CDC42) (down-regulated genes). In T47D, we found only 3 up-regulated genes and 5 down-regulated genes with changes in fold induction greater than 2 fold. Among them, we found chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (up-regulated genes); Glutamate receptor, ionotropic kainite 2, colon carcinoma related protein, insulin-like growth factor binding protein 7 (IGFBP-7), growth differentiation factor 8, KIAA0998. In MCF-7, we identified 83 genes up-regulated and 98 genes down-regulated by Ro25-7328 with changes in fold induction greater than 2 fold. Among them, we found several genes which are involved in cell death, cell growth/maintenance, signal transduction and response to stimulus; i.e., TGF beta2, PKC delta binding protein, Neurophilin 2, Interleukin 6 receptor (up-regulated genes); TGF beta1, Integrin beta 4., Paxillin (PAX), Tumor protein 53 (TP53), C-terminal binding protein 1 (CtBP-1), Mcl-1, Bcl-like1 (down-regulated genes).

In T47D, 16 up-regulated genes and 3 down-regulated genes modulated by LGD1069; 3 up-regulated genes and 5 down-regulated genes were modulated by LGD100268 with changes in fold induction greater than 2 fold. In MDA-MB-231, 335 up-regulated genes and 320 down-regulated genes modulated by LGD1069; 118 up-regulated genes and 432 down-regulated genes were modulated by LGD100268 with changes in fold induction greater than 2 fold. According to the data, we found several interesting genes induced by LGD1069 and LG100268 in MDA-MB-231 such as several

hypothetical proteins, CDC14 cell division cycle 14 homolog A (*S. cerevisiae*), recombination activating gene 2, tumor protein D52, MDM2, ITGA4, ADh1B, NF2 and cathepsin S (for LGD1069), several hypothetical proteins, zinc finger protein 423, cyclin-dependent kinase inhibitor 1C (p57, Kip2), eukaryotic translation initiation factor 5A, transcription factor 4, MDM2, FGF2, GNRH1, and annexin A9 (for LG100268). We will study their functions.

This study could help us to find new preventive/therapeutic target for breast cancer, and may contribute to develop novel molecule which could inhibit breast cancer development.

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Table 1A. Genes Up-regulated by Ro25-7386 in HMEC

probe set	gene	fold change
213872_at	gb:BE465032 /DB_XREF=gi:9510807 /DB_XREF=hv76g09.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, beta 4	26.6
210317_s_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase 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
216971_s_at	plectin 1, intermediate filament binding protein 500kDa	15.17
217211_at	Consensus includes gb:D50604 /DEF=Human beta-cytoplasmic actin (ACTBP9) pseudogene /FEA=CDS /DB_XREF=gi:2094759 /UG=Hs.248007 Human beta-cytoplasmic actin (ACTBP9) pseudogene	14.35
215780_s_at	SET translocation (myeloid leukemia-associated)	12.51
201971_s_at	ATPase, H ⁺ transporting, lysosomal 70kDa, V1 subunit A	11.75
204426_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, alpha 6	10.97
215434_x_at	AG1	10.35
214693_x_at	hypothetical protein MGC8902 /// AG1 /// hypothetical protein DJ328E19.C1.1 /// hypothetical protein LOC200030 /// hypothetical protein LOC348482	10.34
211905_s_at	integrin, beta 4	10.34
201048_x_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_x_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_x_at	stearoyl-CoA desaturase (delta-9-desaturase)	7.3
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) alpha	7.25
201373_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, (alpha)	6.99
213875_x_at	chromosome 6 open reading frame 62	6.93
91816_f_at	ring finger and KH domain containing 1	6.9
200806_s_at	heat shock 60kDa protein 1 (chaperonin)	6.69
214845_s_at	calumenin	6.66
211823_s_at	paxillin	5.75
206665_s_at	BCL2-like 1	5.4
208637_x_at	actinin, alpha 1	5.11
208677_s_at	basigin (OK blood group)	4.66
221499_s_at	syntaxin 16	4.16
209226_s_at	transportin 1	3.9
201752_s_at	adducin 3 (gamma)	3.9
200766_at	cathepsin D (lysosomal aspartyl protease)	3.9
203085_s_at	transforming growth factor, beta 1 (Camurati-Engelmann disease)	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

Table 1B. Genes Down-regulated by Ro25-7386 in HMEC

probe set	gene	fold change
203991_s_at	ubiquitously transcribed tetratricopeptide repeat, X chromosome	-5.32
220568_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, alpha	-4.64
201438_at	collagen, type VI, alpha 3	-4.59
217665_at	Consensus includes gb:AA420614 /FEA=EST /DB_XREF=gi:2094586 /DB_XREF=est:nc62g02.r1 /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, 12kDa	-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
202732_at	protein kinase (cAMP-dependent, catalytic) inhibitor gamma	-3.4
202149_at	neural precursor cell expressed, developmentally down-regulated 9	-3.39
213225_at	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta 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, alpha-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
203741_s_at	adenylate cyclase 7	-2.95
205034_at	cyclin E2	-2.94
204078_at	synaptonemal complex protein SC65	-2.9
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 mu	-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	-2.8
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, 25kDa)	-2.25

Table 2A. Genes Up-regulated by Ro25-7386 in T47D

probe set	gene	fold change
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, alpha)	2.26

Table 2B. Genes Down-regulated by Ro25-7386 in T47D

probe set	gene	fold change
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_a	growth differentiation factor 8	-2.49
210806_at	KIAA0998	-2.3

Table 3A. Genes Up-regulated by Ro25-7386 in MCF-7

probe set	gene	fold change
209909_s_at	transforming growth factor, beta 2	4.94
211430_s_at	immunoglobulin heavy constant gamma 1 (G1m marker)	3.82
213010_at	protein kinase C, delta binding protein	3.76
63825_at	Abhydrolase domain containing 2	3.4
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:3049904 /DB_XREF=ok61b04.s1 /CLONE=IMAGE:1518415 /FEA=EST /CNT=42 /TID=Hs.84285.2 /TIER=Stack /STK=12 /UG=Hs.84285 /LL=7329 /UG_GENE=UBE2I /UG_TITLE=ubiquitin-conjugating enzyme E2I (homologous to yeast UBC9)	3.2
213087_s_at	Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	3.09
217489_s_at	interleukin 6 receptor	3.07
205443_at	small nuclear RNA activating complex, polypeptide 1, 43kDa	3.04
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	2.99
221815_at	Abhydrolase domain containing 2	2.95
212451_at	KIAA0256 gene product	2.93
205363_at	butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1	2.92
212952_at	Consensus includes gb:AA910371 /FEA=EST /DB_XREF=gi:3049661 /DB_XREF=est:ok83h10.s1 /CLONE=IMAGE:1520611 /UG=Hs.16488 calreticulin	2.9
210136_at	Myelin basic protein	2.88
214255_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	neuropilin 2	2.82
204378_at	breast carcinoma amplified sequence 1	2.8
208859_s_at	alpha 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, alpha 4	2.68
218859_s_at	chromosome 20 open reading frame 6	2.65
211626_x_at	v-ets erythroblastosis virus E26 oncogene like (avian) /// v-ets erythroblastosis virus E26 oncogene like (avian)	2.64
214316_x_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	2.6
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=MCF.2 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, beta-induced, 68kDa	2.18
213979_s_at	Consensus includes gb:BF984434 /FEA=EST /DB_XREF=gi:12387246 /DB_XREF=est:602307971F1 /CLONE=IMAGE:4399313 /UG=Hs.239737 C-terminal binding protein 1	2.5
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, beta-induced, 68kDa	2.18

Table 3B. Genes Down-regulated by Ro25-7386 in MCF-7

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) alpha	-5.01
211136_s_at	cleft lip and palate associated transmembrane protein 1	-4.59
204989_s_at	integrin, beta 4	-4.51
213042_s_at	ATPase, Ca++ transporting, ubiquitous	-4.42
216971_s_at	plectin 1, intermediate filament binding protein 500kDa	-4.37
201167_x_at	Rho GDP dissociation inhibitor (GDI) alpha	-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, beta 4	-3.87
211672_s_at	actin related protein 2/3 complex, subunit 4, 20kDa /// actin related protein 2/3 complex, subunit 4, 20kDa	-3.7
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, beta 1 (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	-3.2
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, alpha 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 alpha polypeptide	-2.87
210317_s_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon 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	-2.8
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 500kDa	-2.68
218302_at	presenilin enhancer 2	-2.68
213887_s_at	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa	-2.67
201369_s_at	zinc finger protein 36, C3H type-like 2	-2.59
203392_s_at	C-terminal binding protein 1	-2.5
200796_s_at	myeloid cell leukemia sequence 1 (BCL2-related)	-2.43
206665_s_at	BCL2-like 1	-2.35

Table 4A. Genes Up-regulated by LGD1069 in T47D

probe set	gene	fold change
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
206424_at	cytochrome P450, family 26, subfamily A, polypeptide 1	2.98
202481_at	dehydrogenase/reductase (SDR family) member 3	2.79
211689_s_at	transmembrane protease, serine 2 /// transmembrane protease, serine 2	2.61
213629_x_at	metallothionein 1F (functional)	2.32
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	2.32
208581_x_at	metallothionein 1X	2.31
210827_s_at	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	2.28
217165_x_at	metallothionein 1F (functional)	2.23
204326_x_at	metallothionein 1X	2.19
206461_x_at	metallothionein 1H	2.15
204470_at	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	2.14
204745_x_at	metallothionein 1G	2.13
217028_at	chemokine (C-X-C motif) receptor 4	2.04
212185_x_at	Consensus includes gb:NM_005953.1 /DEF=Homo sapiens metallothionein 2A (MT2A), mRNA. /FEA=CDS /GEN=MT2A /PROD=metallothionein 2A /DB_XREF=gi:5174763 /UG=Hs.118786 metallothionein 2A /FL=gb:NM_005953.1	2.02
211456_x_at	gb:AF333388.1 /DB_XREF=gi:13310411 /FEA=FLmRNA /CNT=1 /TID=Hs.326774.0 /TIER=FL /STK=0 /UG=Hs.326774 /DEF=Homo sapiens metallothionein 1H-like protein mRNA, complete cds. /PROD=metallothionein 1H-like protein /FL=gb:AF333388.1	2.01

Table 4B. Genes Down-regulated by LGD1069 in T47D

probe set	gene	fold change
207437_at	neuro-oncological ventral antigen 1	-3.04
210806_at	KIAA0998	-2.33
202989_at	regulator of G-protein signalling 1	-2.13

Table 5A. Genes Up-regulated by LG100268 in T47D

probe set	gene	fold change
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, alpha)	2.26

Table 5B. Genes Down-regulated by LG100268 in T47D

probe set	gene	fold change
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	-2.3

Table 6A. Genes Up-regulated 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. /FEA=mRNA /GEN=PRO1866 /PROD=hypothetical protein PRO1866 /DB_XREF=gi:8924091 /UG=Hs.283031 hypothetical protein PRO1866 /FL=gb:AF119858.1 gb:NM_018510.1	30.5
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-Ec-bioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2393-2682 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 dethiobiot	9.76
205386_s_at	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	9.65
216119_s_at	chromosome 20 open reading frame 28	9.42
AFFX-BioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 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 dethiobiot	9.32
209613_s_at	alcohol dehydrogenase IB (class I), beta polypeptide	8.85
AFFX-r2-Ec-bioB-3_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 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 dethiobiot	8.78
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	7.08
205524_s_at	hyaluronan 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	6.7
215526_at	Consensus includes gb:AL050145.1 /DEF=Homo sapiens mRNA; cDNA DKFZp586C2020 (from clone DKFZp586C2020). /FEA=mRNA /DB_XREF=gi:4884356 /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_x_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	5.4
216795_at	CDNA: FLJ23194 fis, clone REC00490	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_x_at	MCF.2 cell line derived transforming sequence-like 2	5.11
216775_at	ubiquitin specific protease 53	4.9
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	4.7
AFFX-r2-Ec-bioD-3_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 5312-5559 of gb:J04423.1, not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	4.66
220564_at	chromosome 10 open reading frame 59	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-	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 5286-5570 of gb:J04423.1,	4.49

3_at	not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	
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_x_at	neuronal thread protein AD7c-NTP	4.32
214767_s_at	heat shock protein, alpha-crystallin-related, B6	4.31
216697_at	Triple functional domain (PTPRF interacting)	4.28
222341_x_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	neurofibromin 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 dethiobiot	3.83
211315_s_at	calcium channel, voltage-dependent, alpha 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

Table 6B. Genes Down-regulated by LGD1069 in MDA-MB-231

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=GLCSB09 /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, Brigham & Women's Genetics 1357 expressed [Mus musculus]	-5.43
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:L48784 /DEF=050 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 azoospermia-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 68kDa	-4.2
202648_at	gb:BC000023.1 /DB_XREF=gi:12652562 /FEA=FLmRNA /CNT=966 /TID=Hs.298262.0 /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 (gamma), 2-oxoglutarate dioxygenase (gamma-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, 65kDa)	-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	-3.8
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 1032F1	-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_x_at	cathepsin S	-3.42
204681_s_at	Rap guanine nucleotide exchange factor (GEF) 5	-3.35
222227_at	Zinc finger protein 236	-3.35
207465_at	PRO0628 protein	-3.34

Table 7A. Genes Up-regulated by LG100268 in MDA-MB-231

probe set	gene	fold change
219948_x_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	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_x_at	trinucleotide repeat containing 9	7.58
203603_s_at	zinc finger homeobox 1b	5.77
205386_s_at	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	5.2
205419_at	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	4.18
216978_x_at	Consensus includes gb:U50277.1 /DEF=Human breast cancer suppressor element Ishmael Upper CP1 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 CP	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_s_at	gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	3.52
205651_x_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_x_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	3.1
206381_at	sodium channel, voltage-gated, type II, alpha 2	3.09
212182_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	2.98
215112_x_at	MCF.2 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	2.8
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-Ec-bioB-M_at	E. coli /GEN=bioB /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 2393-2682 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 dethiobiot	2.55
216775_at	ubiquitin specific protease 53	2.54
206201_s_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	2.53
AFFX-BioDn-5_at	E. coli /GEN=bioD /DB_XREF=gb:J04423.1 /NOTE=SIF corresponding to nucleotides 4980-5256 of gb:J04423.1, not 100% identical /DEF=E.coli 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC pro	2.48
216894_x_at	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	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

Table 7B. Genes Down-regulated by LG100268 in MDA-MB-231

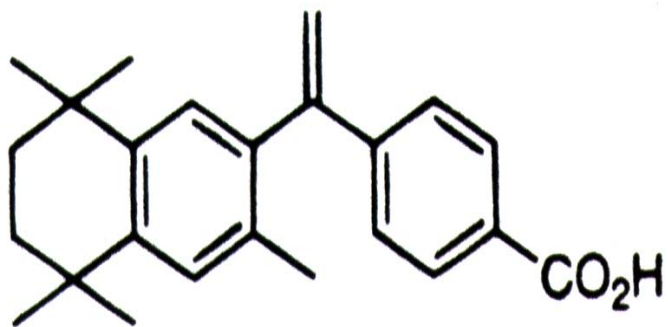
probe set	gene	fold change
217237_at	Zinc finger protein 423	-78.6
215014_at	Consensus includes gb:AL512727.1 /DEF=Homo sapiens mRNA; cDNA DKFZp547P042 (from clone DKFZp547P042). /FEA=mRNA /DB_XREF=gi:12224870 /UG=Hs.232127 Homo sapiens mRNA; cDNA DKFZp547P042 (from clone DKFZp547P042)	-17.74
213753_x_at	eukaryotic translation initiation factor 5A	-7.65
212382_at	Transcription factor 4	-5.74
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.	-5.58
211712_s_at	annexin A9 /// annexin A9	-5.49
209227_at	tumor suppressor candidate 3	-5.11
216917_s_at	synaptonemal complex protein 1	-4.82
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.	-4.31
210697_at	zinc finger protein 257	-4.11
215013_s_at	ubiquitin specific protease 34	-3.97
209657_s_at	heat shock transcription factor 2	-3.96
221009_s_at	angiopoietin-like 4	-3.9
205612_at	multimerin 1	-3.79
207613_s_at	calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	-3.55
37232_at	KIAA0586	-3.38
AFFX-HUMRGE/M10098_3_at	signal recognition particle 68kDa	-3.37
204422_s_at	fibroblast growth factor 2 (basic)	-3.33
220638_s_at	Cas-Br-M (murine) ecotropic retroviral transforming sequence c	-3.32
208098_at	olfactory receptor, family 12, subfamily D, member 3 /// olfactory receptor, family 12, subfamily D, member 3 /// olfactory receptor, family 5, subfamily V, member 1 /// olfactory receptor, family 5, subfamily V, member 1	-3.29
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	-3.25
208453_s_at	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	-3.2
207485_x_at	butyrophilin, subfamily 3, member A1	-3.18
211032_at	COBL-like 1 /// COBL-like 1	-3.11
220619_at	chromodomain helicase DNA binding protein 7	-3.04
209318_x_at	pleiomorphic adenoma gene-like 1	-3
201547_at	Jumonji, AT rich interactive domain 1B (RBP2-like)	-2.99
206996_x_at	calcium channel, voltage-dependent, beta 1 subunit	-2.98
220114_s_at	stabilin 2	-2.95
216709_at	Hypothetical gene supported by BC013370; BC034583	-2.93
203555_at	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	-2.92
213267_at	KIAA1117	-2.91
201122_x_at	eukaryotic translation initiation factor 5A	-2.89
213495_s_at	gb:AW166067 /DB_XREF=gi:6397592 /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=RRBP1 /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_x_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 kappa constant	-2.79
214001_x_at	gb:AW302047 /DB_XREF=gi:6711724 /DB_XREF=xr52f08.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_x_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

Table 8. Forward and reverse primers for amplification of targeted genes with real-time RT-PCR

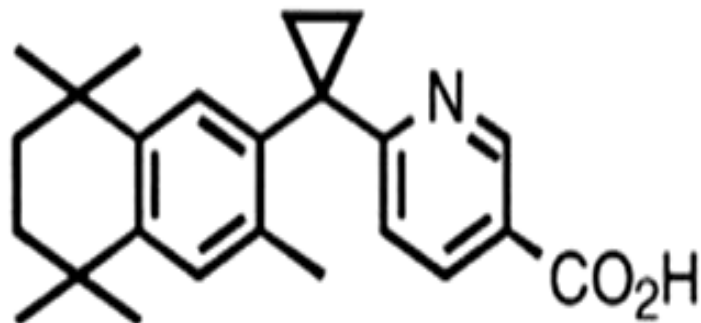
Target genes	Forward primers	Reverse primers
BAX	5'-TGGAGCTGCAGAGGATGATTG-3'	5'-GAAGTTGCCGTCAGAAAACATG-3'
E-cadherin = CDH1	5'-CACTGCCAACTGGCTGGAG-3'	5'-GGGTTAGCTCAGCAGTAAAG-3'
FOXO3A=FKHRL1	5'-TCAATCAGAACTTGCTCCACCA-3'	5'-GGACTCACTCAAGCCCATGTTG-3'
Integrin alpha 6	5'-TTTCCCGTTTCTTTCTTGAGTTGT-3'	5'-TGGAAAAGGTAAGTTGTGAGCCA-3'
Integrin beta 4	5'-TTCCAAATCACAGAGGAGAC-3'	5'-CTTGAGGTTGTCCAGATCAT-3'
PXN = paxillin	5'-TGGCTTCGCTGTCGGATTTC-3'	5'-GTCAAGGGCTGTCACCACTTTATC-3'
PTEN	5'-AGAGCGTGCAGATAATGACAAG-3'	5'-GGATCAGAGTCAGTGGTGTGTCAG-3'
STAT	5'-CTGCTGCGGTTCAGTGAGAG-3'	5'-CCAAGTGAAAGTGACCCCTCC-3'
Collagen type VI alpha 3	5'-CTGGGCAGACATACCATGTG-3'	5'-GCAAGTCCTTCGTCTTTTCG-3'

SUPPORTING DATA

Molecular Structure of LGD1069 and LG100268



LGD1069



LG100268

Figure 1

**Expression of RXR alpha in breast cells (western blot analysis) –
normal (HMEC) and breast cancer cells**

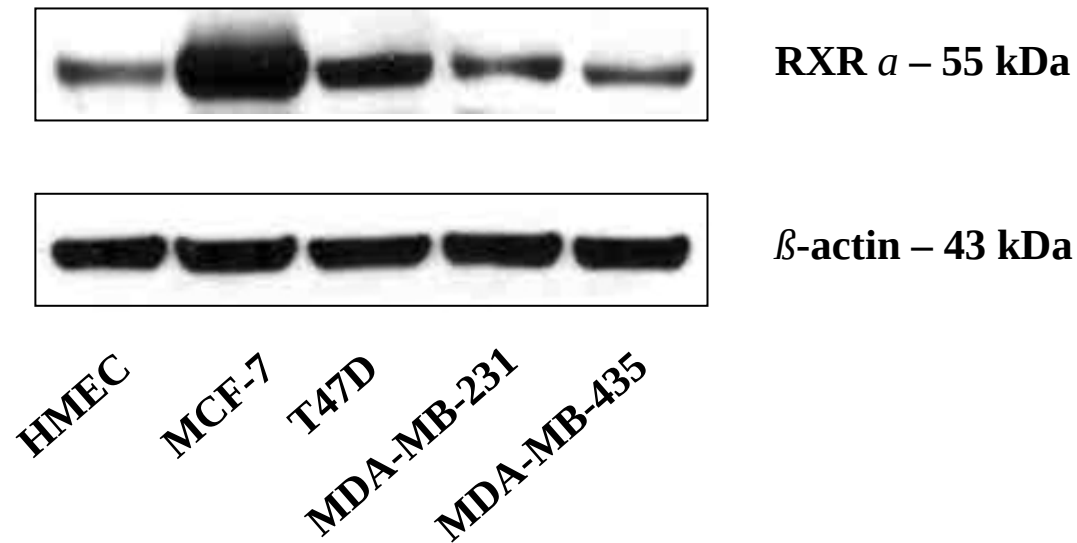


Figure 2

Cell Proliferation Assay with RXR alpha ligand (Ro25-7386)

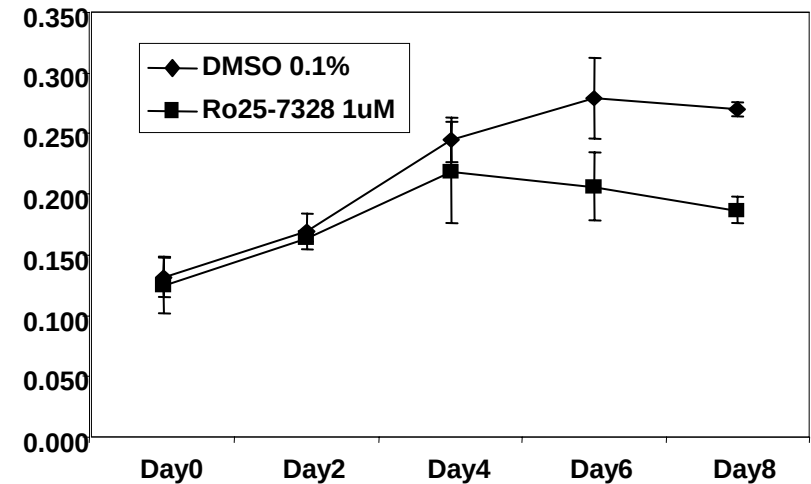
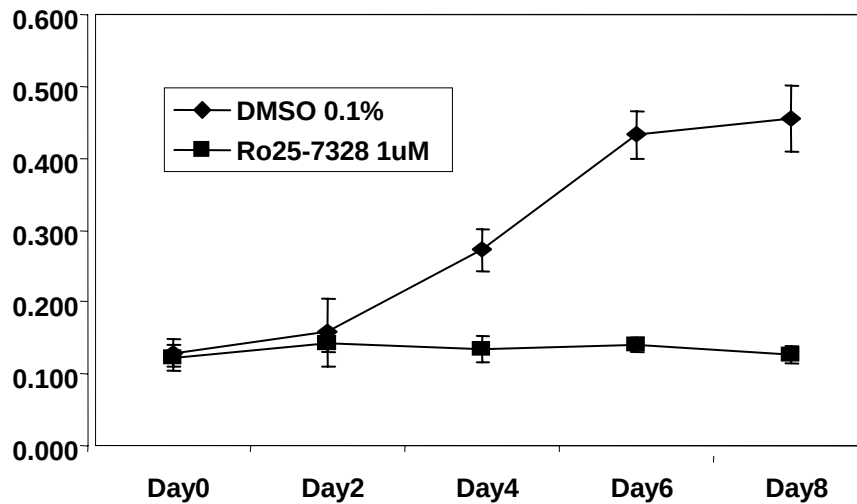
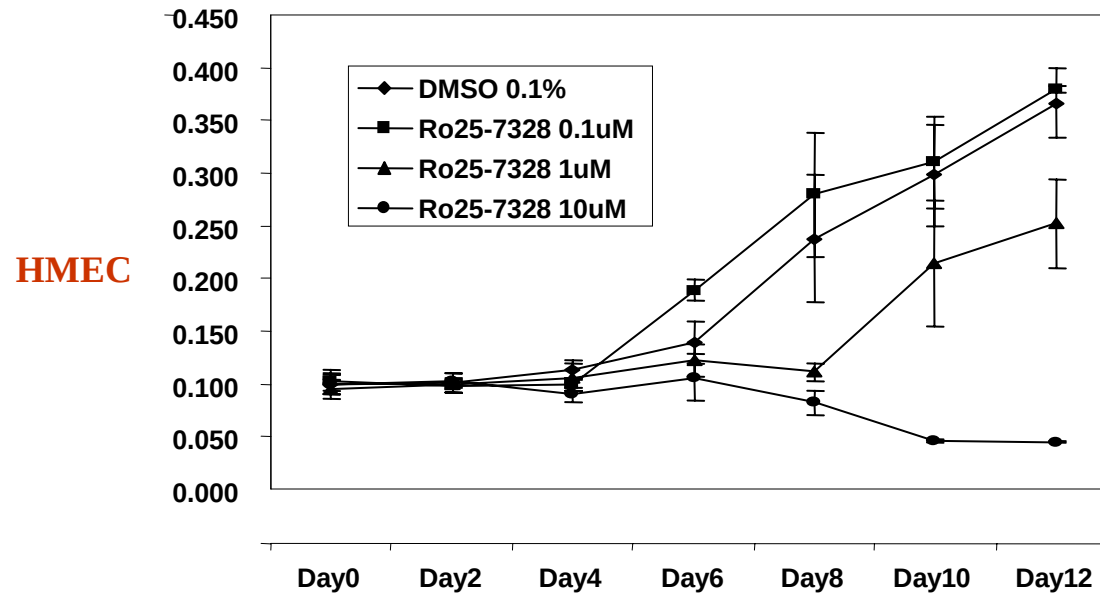


Figure 3

The effect of LGD1069 and LG100268 on the growth of HMEC

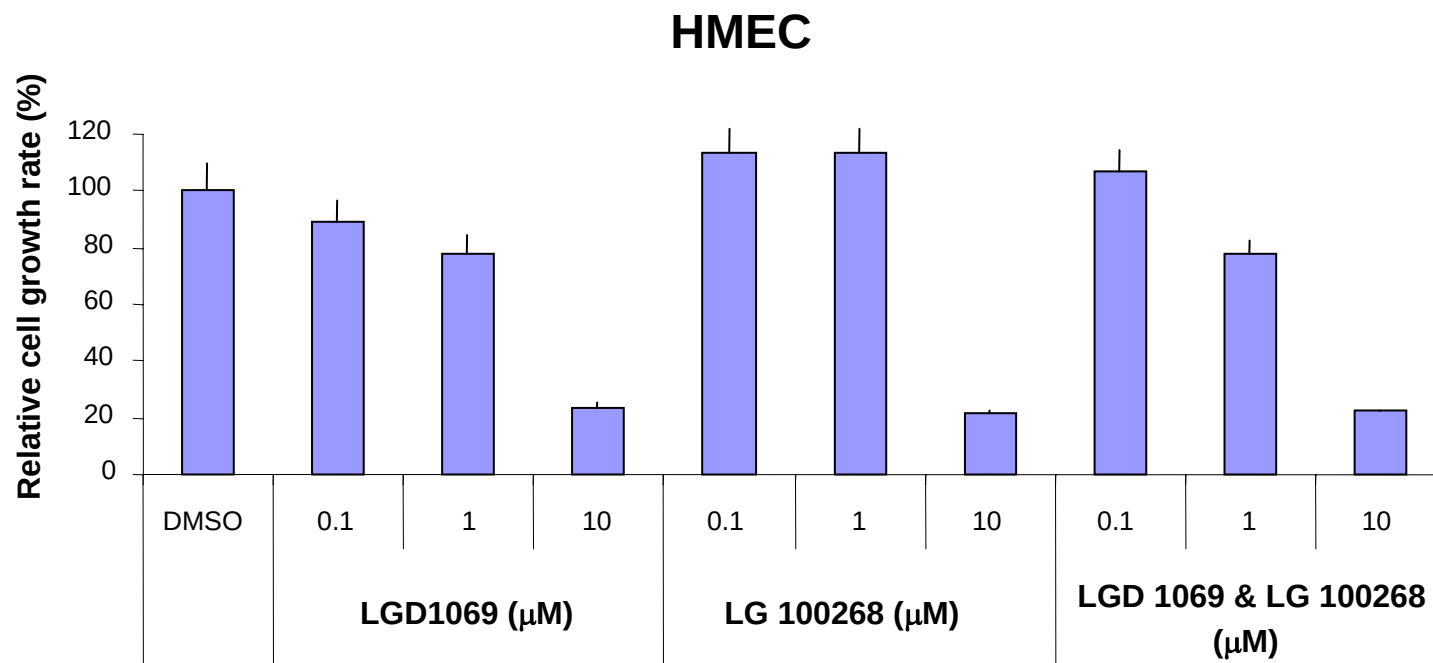


Figure 4

The effect of LGD1069 and LG100268 on the growth of MCF-7 and T47D

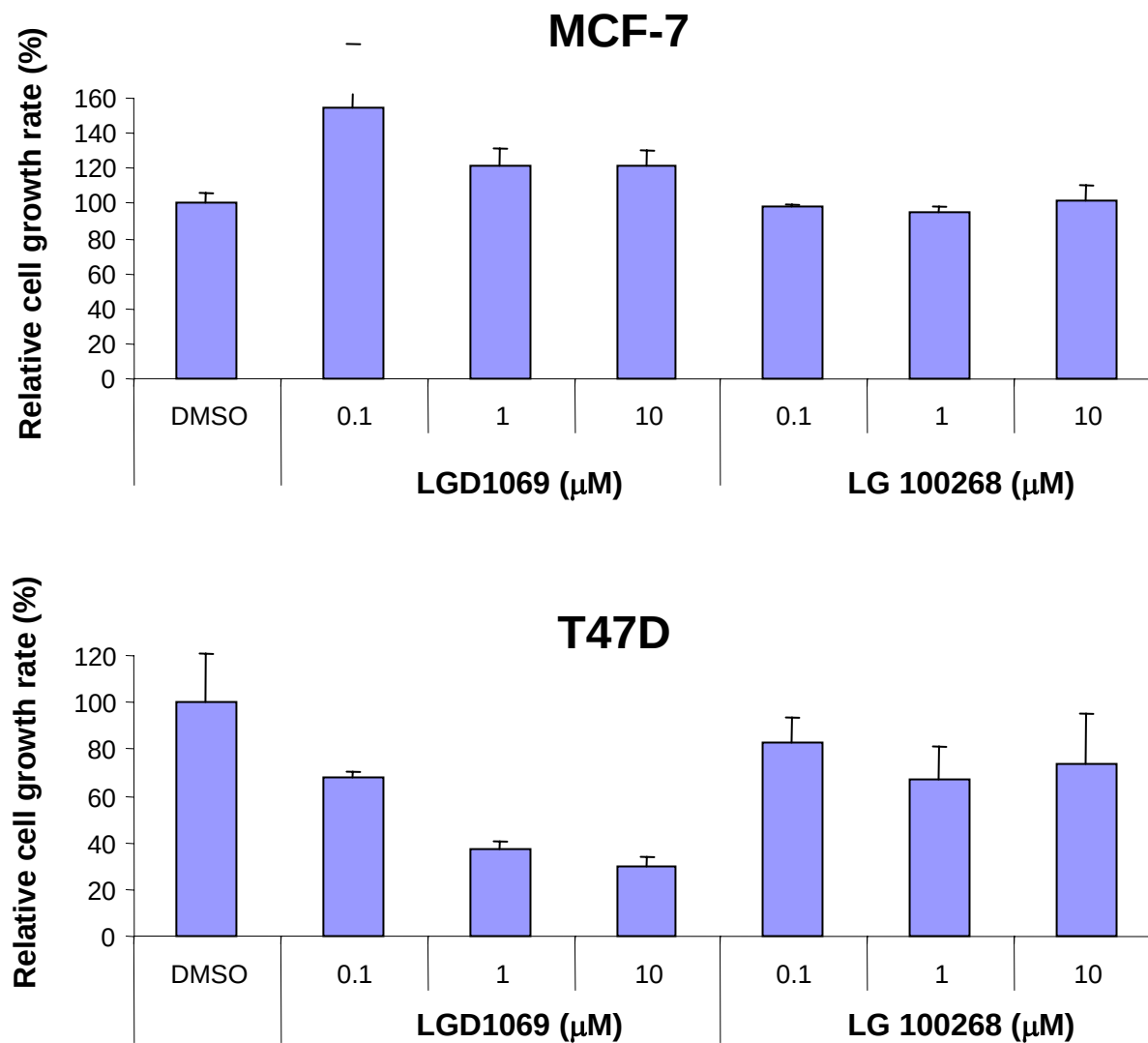


Figure 5

The effect of LGD1069 and LG100268 on the growth of MDA-MB-231 and MDA-MB-435

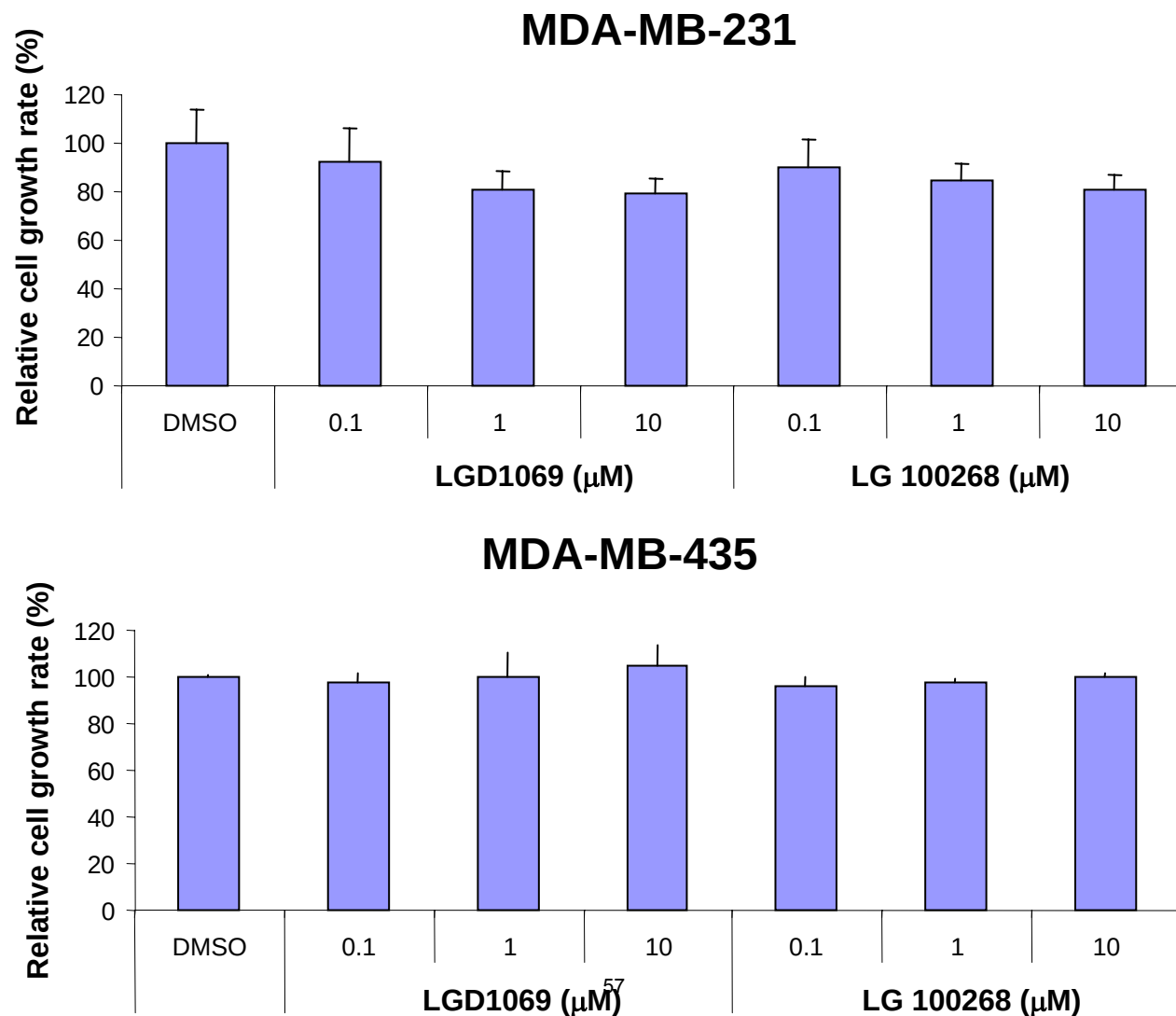


Figure 6

Cell Growth Suppressive Activity

	HMEC	MCF-7	T47D	MDA-MB-231
Ro25-7386	●	●	●	X
LGD1069	●	X	●	●
LG100268	●	X	X	●

Affymetrix Microarray Data

	HMEC	MCF-7	T47D	MDA-MB-231
Ro25-7386	●	●	●	X
LGD1069	X	X	●	●
LG100268	X	X	X	●

Figure 7

mRNA levels of RXR α -regulated genes in HMEC (measurement by real-time RT-PCR)

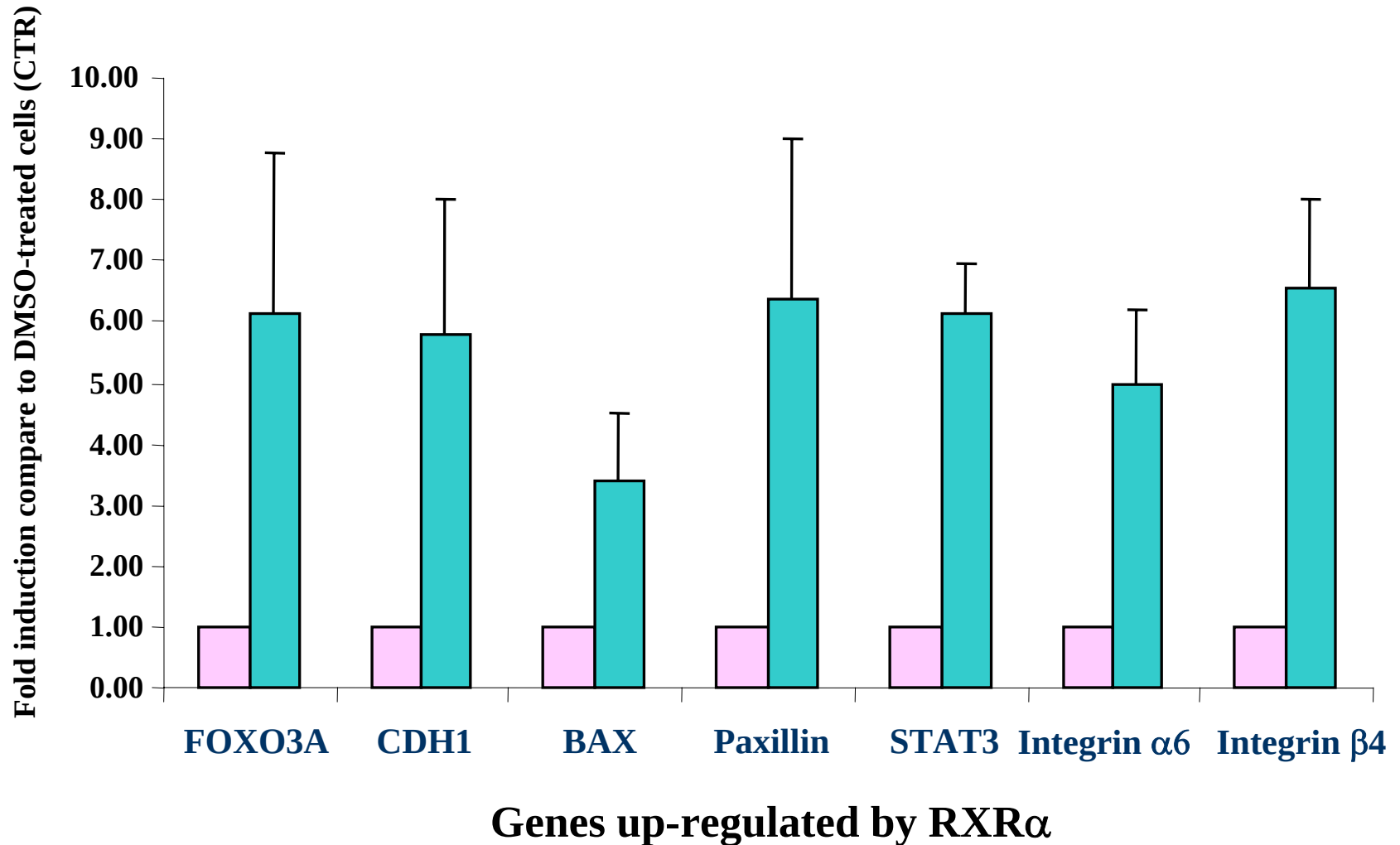


Figure 8

**Protein levels of RXR α -regulated genes in HMEC
(measurement by Western blot analysis)**

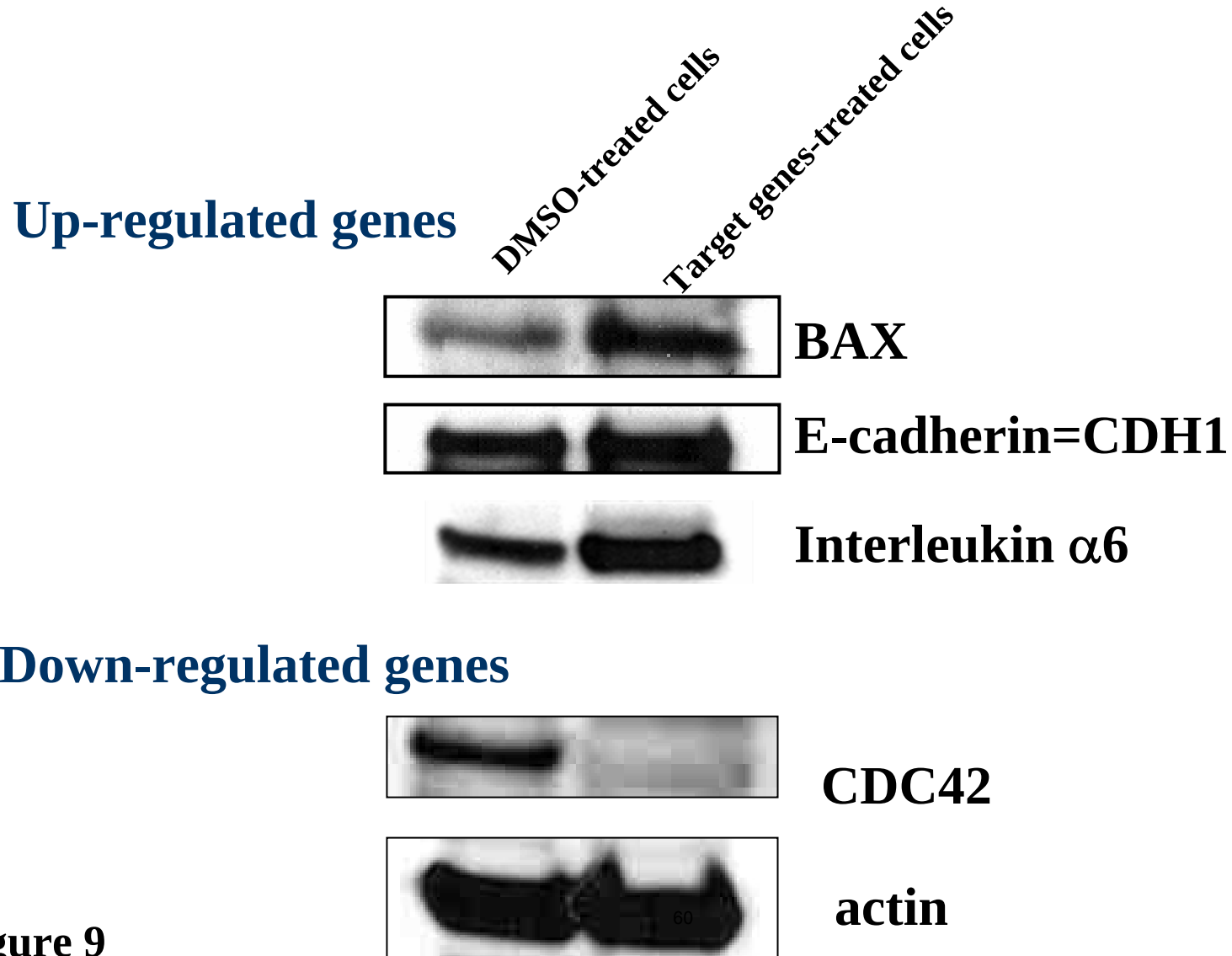


Figure 9