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14. ABSTRACT
Estrogen is essential for normal growth and development of the female reproductive system and breast. Lifetime exposure to estrogen may affect a woman's risk for breast cancer. Approximately 70% of breast cancers are estrogen receptor (ER) positive and adjuvant antiestrogen therapy is considered as a primary therapy for those cancer patients. In the past 2 decades, selective estrogen receptor modulators (SERMs), especially tamoxifen, have been clinically used. Although tamoxifen is a powerful blocking drug for estrogen receptor in breast cancer tissue, it is also known as a weak estrogen signal stimulator in other organs such as liver, bone and uterine. As such it is now believed that tamoxifen can slightly increase the risk of uterine cancer. While tamoxifen has been clinically used as the first line therapeutic drug, many researchers, including our research group, were developing drugs that would inhibit the production of estrogen. Estrogen is produced by the ovaries and other tissues of the body using an enzyme called aromatase. Once women have reached menopause, the ovaries no longer produce estrogen. Therefore, particularly in postmenopausal women with breast cancer, estrogen production in the breast tissue can regulate the cancer growth. Currently, the third generation of aromatase inhibitors (AIs), such as exemestane (Aromasin®), anastrazole (Arimidex®) and letrozole (Femera®), are approved by US. Food and Drug Administration and clinically used for those estrogen sensitive breast cancer patients with postmenopausal women.

15. SUBJECT TERMS
Aromatase, tissue specific delivery, siRNA, liquid cationic liposom, lipid nanoparticles

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Introduction

Estrogen is essential for normal growth and development of the female reproductive system, including breast tissue, and lifetime exposure to estrogen may affect a woman's risk for breast cancer. Estradiol-17β (E_2) is the most potent endogenous estrogen and is biosynthesized from androgens by a cytochrome P450 enzyme complex called aromatase. The *CYP19* gene, which encodes for aromatase, consists of 10 exons, and its full length cDNA of 3.4 kb encodes a protein of 503 amino acids. The coding region is approximately 30 kb in size, and the regulatory region is ~93 kb. The regulation of aromatase is complex in various tissues, and several tissue-specific promoter regions have been identified upstream of the *CYP19* gene coding region. Although no protein isoforms are generated, the mRNA variants resulting from alternative promoters have different tissue specificity. These tissue-specific promoters include promoter PI.1, PI.3, PI.4, PI.6, PI.7, and PII. The PII promoter is utilized in normal ovary and breast cancer tissues. Promoter PI.4 is the primary promoter used in normal adipose tissue. Promoter PI.3 is also present in adipose tissues such as normal breast tissue, and PI.3 is elevated in breast cancer tissues. The long term goal of this research is to develop novel therapeutic interventions targeting tissue-specific expression of aromatase. siRNAs are oligonucleotide duplexes that can be designed to target the CYP19 promoter regions. A major challenge to therapeutic application of siRNA is the lack of an efficient in vivo delivery system. Targeted nanoparticles can protect siRNA against nuclease degradation and facilitate tumor cell specific delivery of siRNA in vivo. Therefore, another objective of this project is to design, synthesis, and characterization of lipid nanoparticles for siRNA delivery.

The specific aims of this grant are (1) to regulate the aromatase expression in tissue specific manner by using a functional genomic approach and (2) To examine a tissue specific aromatase RNAi delivery system *in vivo*. This research project involves synergistic collaboration of two research groups. The first research group is directed by Dr. Robert Brueggemeier and focuses on aromatase biochemistry, siRNA molecules, and breast cancer cell lines. This research is fund under the award number **W81XWH-08-1-0521**. The second research group is directed by Dr. Robert Lee and examines nanoparticle formulation of siRNAs, stability and bioavailability, and in vivo testing of novel siRNA formulations. This research is fund under the award number **W81XWH-08-1-0610**.

Body

Task 1: To regulate the aromatase expression in tissue specific manner by using a functional genomic approach

The following segment is being performed by Dr. Brueggemeier's laboratory
Months 1-6: Establishment of Tetracycline-regulatable aromatase expression system.

a. *Construct the Tetracycline- regulatable expression vectors containing aromatase.*

b. *Transfect the plasmids into MCF-7 cells to establish transfected cell lines*

c. *Ensure the reproducibility and the regulation of tetracycline of the transfected cell lines using Western blotting analysis.*

Months 1-4: construction of siRNA library.

d. *Designing the specific siRNA for each group.*

e. *Synthesis of siRNA*

Month 7-10: in vitro characterization of established cell line described above.

f. *mRNA and protein expressions will be analyzed. Aromatase enzyme activities will be measured.*

Both the Dr. Brueggemeier's and the Dr. Lee's laboratories are contributing on the following segment. The 1st year report for W81XWH-08-1-0521 (Brueggemeier) contains more details.

Months 10-14: in vivo characterization of established cell line described above.

g. *Inoculate the transfected or non-transfected cells into mice orthotopically.*

Ten mice were inoculated with **MCF-7 aro** suspended in Matrigel and tumor growth was observed for 60 days. No tumor formation was observed within 60 days.

Ten mice were inoculated with **MCF-7 I.3a** suspended in Matrigel and tumor growth was observed for 60 days. No tumor formation was observed within 60 days.

h. *Implant placebo pellet into control group.*

Ten mice were inoculated with **MCF-7 aro** suspended in Matrigel and tumor growth was observed for 60 days. No tumor formation was observed within 60 days.

Ten mice were inoculated with **MCF-7 I.3a** suspended in Matrigel and tumor growth was observed for 60 days. No tumor formation was observed within 60 days.

i. *Implant testosterone pellet into other host animals.*

Ten mice were inoculated with **MCF-7 aro** suspended in Matrigel and tumor growth was observed for 60 days. Only 2 mice carried xenografted tumor after 60 days.

Ten mice were inoculated with **MCF-7 I.3a** suspended in Matrigel and tumor growth was observed for 60 days. Only 2 mice carried xenografted tumor after 60 days.

The testosterone pellet used was 7.5mg per pellet for 60 days slow release or approximately 5mg/kg/day. Alternative approaches for enhancing the maintenance and growth of the xenografts will be explored in year 2.

Alternative approaches for enhancing the maintenance and growth of the xenografts will be explored in year 2.

j. Aromatase expression will be controlled by administration of tetracycline.

n/a (not applicable for year 1)

k. Monitor the tumor formation and determine the optimum condition

n/a

<u>Months 17-19:</u> Testing the siRNA-LN formlation

l. Inoculate the transfected cells into mice orthotopically.

In progress as described in the section f.

m. Monitor the tumor progression by measuring the tumor size three-dimensionally.

In progress as described in the section f.

n. siRNA-LN injection upon verifying tumor formation.

n/a

o. Necropsy at day 60.

n/a

p. aromatase protein will be localized using immunohistochemical staining

n/a

q. Perform real-time quantitative RT- PCR on aromatase, Presenilins (PS2), progesterone receptor (PR), and others.

n/a

Months 20-24: Data analysis and finish up

r. Data analysis and preparing for final report.

n/a

Task 2: To examine a tissue specific aromatase RNAi delivery system *in vivo*

The following segment is being performed by Dr. Lee's laboratory

Months 1-9: Development of optimum formulation of LN for the siRNA.

s. Formulation of siRNA(control)-LN

Synthesis of siRNA-LN formulations

siRNAs are potent effectors of post-transcriptional gene silencing. The purpose of this study was to develop novel cationic lipid nanoparticles for delivery of siRNA to breast cancer.

siRNA-LNs were prepared by an ethanol injection method: Cationic lipids were dissolved in ethanol, and then slowly added to siRNA solution under vortexing at an siRNA-to-lipid ratio of 1:10 (w/w). The preparation was then dialyzed against HEPES-buffered saline (HBS, pH 7.5), using a MWCO 10,000 Da DispoDialyzer® (Spectrum Labs., Rancho Dominguez, CA), to remove the ethanol. The composition of the LNs was varied to optimize the formulation for physical chemical and biological activity. The following is a Table summarizing formulations studied:

Table I. Compositions of LNs synthesized

Formulations	Cationic lipid	Co-lipid	PEGylation	Condensing Agent
1	DDAB	Cholesterol	TPGS	PEI2k
2	DDAB	Cholesterol	TPGS	Protamine
3	DOTAP	Cholesterol	TPGS	PEI2k
4	DOTAP	Cholesterol	TPGS	Protamine
5	DC-Chol	Egg PC	TPGS	PEI2k
6	DC-Chol	Egg PC	TPGS	Protamine

Of these formulations, formulation 1 was found to be the most biologically active and therefore used in subsequent studies.

t. Characterization of siRNA(control)-LN

Particle size and distribution of the siRNA-LN were analyzed on a NICOMP Particle Sizer Model 370 (Particle Sizing Systems, Santa Barbara, CA). Zeta potential of the

LNs was determined on a zeta potential analyzer ZetaPlus (Brookhaven Instruments Corp., Worcestershire, UK). A typical preparation has a particle size of 67.8 ± 3.5 nm and zeta potential of +22.1 mV.
The transfection activity of the LNs was analyzed in vitro using a reporter gene target. A GFP-stable-transfected cell line was created by introduction of the plasmid phrGFP-N1 into MCF-7 cells. The resulting cell line, MCF-7-phrGFP s11, was used in further studies to determine the transfection efficiency of the siRNA-LNs. The optimized formulation will then be used for siArom delivery and then for the siRNA for exon I.3 specific sequence. Stably transfected cell lines were also generated using MDA-MB-231 and SK-Br-3 cell lines.

In vitro cellular uptake

The phrGFP transfected MCF-7, MDA-MB-231 and SK-Br-3 cells were seeded in 6-well plates 18 hrs before experiments. The Cells were treated with siRNA-LN formulations at 37 ^{o}C for 4 hrs. The cells were further incubated for 44 hrs. The Cells were then washed three times with PBS followed by incubation with 980 ul 8.0 mM NaOH solution at 60^{o}C for 20 min, and then 20 ul 2.0 mM Hepes buffer was added. Fluorescence intensity of 100 ul cell lysates were determined by a micro plate fluorescence reader (λex: 485 nm, λem: 535 nm). The protein concentrations of the samples were determined using a protein kit (Micro BCA protein assay kit, Pierce). Alternatively, cells were washed, and fixed with 4% paraformaldehyde. The fluorescence of phrGFP cell was then measured by flow cytometry.

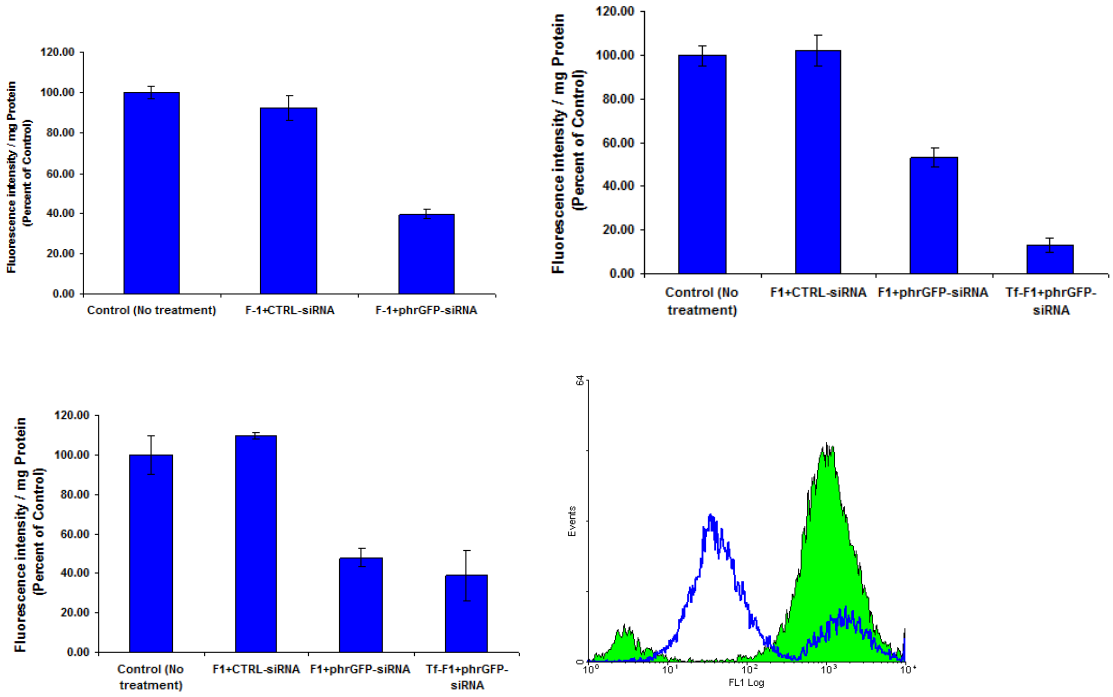

Fig. 1. Comparison of siRNA delivery and silencing efficiency of phrGFP-siRNA LN formulations and control after 48 hrs of transfection in stably phrGFP transfected MCF-7, MDA-MB-231, and SK-BR-3 cells by (A) in vitro cellular uptake by fluorometry and (B) by flow cytometry.

Target silencing measured by qRT-PCR

Total RNA was extracted from the cells using Trizol reagent and cDNA was synthesized with a SuperScript III kit (Invitrogen) according to the manufacture's protocol. RNA concentration was calculated by absorbance at 260 nm. 2 ug RNA were used for cDNA synthesis. RT reaction was performed by using random hexamers (Invitrogen, P/N 18080-051) and SuperScript III reverse transcriptase. For, PCR, 2 ul cDNA solution were used. PCR for phrGFP was performed with the following primers: forward CAG GAG ACA TGA GCT TCA AGG TGA A and reverse TTC ACC TTG AAG CTC ATG TCT CCTG. Primers for 36B4 as internal standard were forward AAACTGCTGCCTCATATCCG and reverse TTTCAGCAAGTGGGAAGGT. PCR was performed for 40 cycles under the following conditions: 95 ^{o}C, 15s (denaturation); 60 ^{o}C 60s.

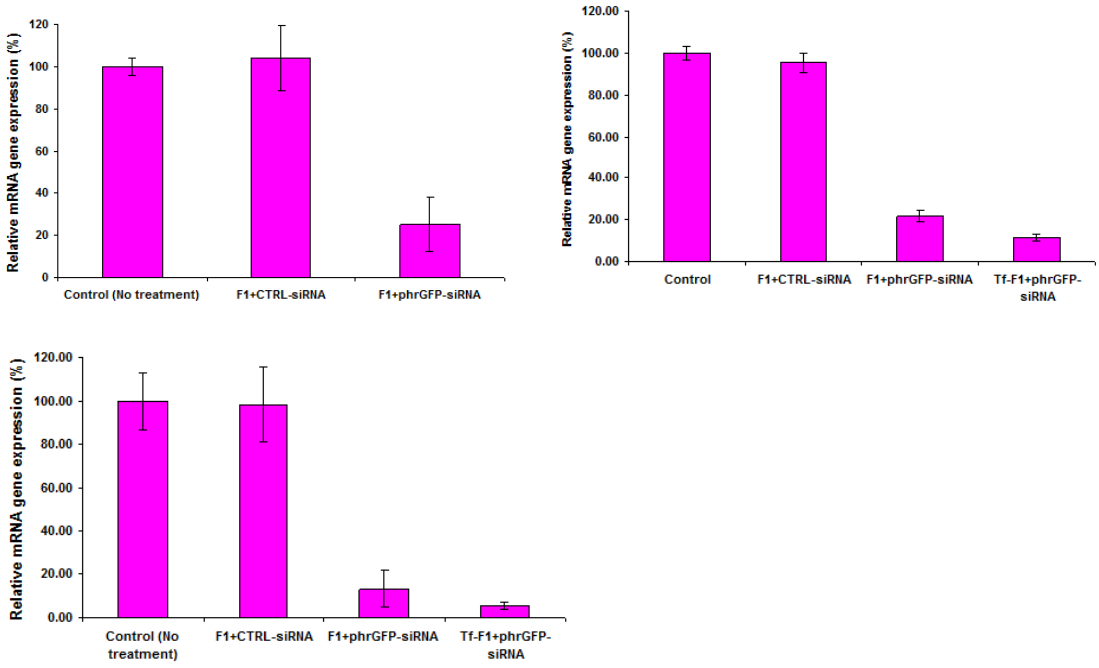

Fig. 2. Comparison of siRNA delivery and silencing efficiency of phrGFP-siRNA LN formulation and control at 48 hrs post transfection in stably phrGFP transfected MCF-7, MDA-MB-231 and SK-BR-3 cells by qRT-PCR.

Aromatase enzyme assay

Aromatase activity was determined by measuring the amount of [^{3}H]-H_2O released with the conversion of [1β-^{3}H]androstenedione into estrone. The cells were cultured in a 6-well plate in DMEM/F-12 supplemented 10(v/v) % FBS in the presence or absence of siRNA formulations for 4 hours. Culture medium was replaced with fresh medium and incubated for another 44 hours. The cells were then incubated with [1β-^{3}H] androstenedione for an additional 8 hours. Steroids in the medium were extracted with ether and centrifuged. The aqueous phase was then mixed with 1 % charcoal / 0.5 % Dextran and incubated for 10 minutes at 45^{o}C. The mixture was subsequently centrifuged, and the supernatant was added to 5 ml scintillation fluid and assayed for radioactivity. The amount of radioactivity in [^{3}H]-H_2O was normalized with the total DNA concentration determined using HOECHST33258 fluorescent day with known amount of calf thymus DNA as the standard.

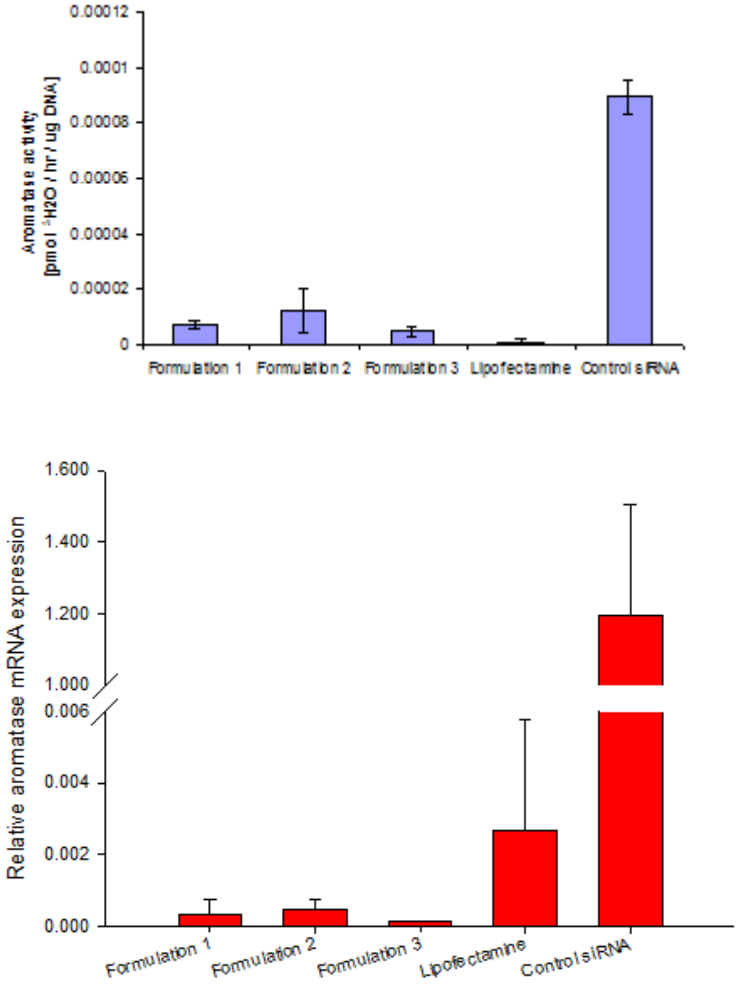

Fig.3. Comparison of aromatase activity and aromatase gene down-regulations of Aro-siRNA formulations by Enzyme assay and by qRT-PCR.

Both Dr. Brueggemeier's and Dr. Lee's laboratories are contributing on the following segment:

Months 6-14: Characterization of siRNA(control)-LN in vivo

u. siRNA(control)-LN distribution in vivo

v. siRNA(control)-LN Pharmacokinetic study in vivo

w. siRNA(control)-LN toxicity study in vivo

x. siRNA(control)-LN standard drug effect profiling study in vivo

Initiation of this part (u – x) of the project is pending final formulation optimization and characterizations, as described in section s and t. In addition, appropriate detection methods for analysis of blood samples from in vivo studies are now being developed.

Months 15-17: Characterization of siRNA(control)-LN

y. siRNA(aromatase)-LN distribution in vivo

Studies to be performed in yr 2.

z. siRNA(aromatase)-LN Pharmacokinetic study in vivo

Studies to be performed in yr 2.

aa. siRNA(aromatase)-LN toxicity study in vivo

Studies to be performed in yr 2.

bb. siRNA(aromatase)-LN standard drug effect profiling study in vivo

Studies to be performed in yr 2.

Key Research Accomplishments

- An optimum siRNA-LN formula and its transfection condition was established.
- Three breast cancer cell lines stably transfected with phrGFP-N1 plasmid were developed.
- A formulation screening assay method was developed by using phrGFP-N! transfected breast cancer cell lines.

Reportable Outcomes

There are a manuscript accepted for publication and four presentation abstracts at a scientific conferences.

"Liposomal siRNA Delivery to Silence Aromatase for Breast Cancer Treatment"
Megan Lynn Cavanaugh , L. James Lee , Robert J. Lee , Yasuro Sugimoto and Robert W. Brueggemeier,
2008 Annual Meeting of American Institute of Chemical Engineers
November 16-21, 2008, Loews Philadelphia Hotel,
Philadelphia Marriott Downtown & Pennsylvania Convention Center
Philadelphia, PA

"Selective Regulation of Aromatase Expression for Drug Discovery"
Robert W. Brueggemeier, Bin Su, Michael V. Darby, and Yasuro Sugimoto,
2008 International Aromatase Conference, October 13 to 17, 2008
Shanghai, China

"Liposomal siRNA Delivery to Silence Aromatase"
Yasuro Sugimoto, Megan Cavanaugh, L. James Lee, Robert J. Lee and Robert W. Brueggemeier
2008 International Aromatase Conference, October 13 to 17, 2008
Shanghai, China

"Liposomal siRNA delivery to silence aromatase"
Yasuro Sugimoto, Megan L. Cavanaugh, Longzhu Piao, Robert J. Lee, L. James Lee, Robert W. Brueggemeier. AACR 100th Annual Meeting 2009, April 18-22, 2009 in Denver, CO

UPDATE since September, 2009

In vivo studies have been initiated. This include generation of a xenograft model of nude mice implanted with GFP transfected breast cancer, and characterization of in vivo silencing of the GFP gene determined using IVIS in vivo imaging of the mice.

Conclusion

Substantial progress has been made towards accomplishing the goals of the project. siRNA LN formulations have been synthesized and analyzed for biological activity. Stable tranfected cell lines were generated to enable efficient determination of siRNA delivery activity. An excellent formulation has been developed that efficiently down regulated the targeted gene in vitro. Year 2 of this project will be focused on in vivo characterization of the nanoparticles, including pharmacokinetic studies and target down regulation studies using siRNA against the GFP reporter gene and the aromatase target gene.

Appendices

I. “Liposomal siRNA Delivery to Silence Aromatase for Breast Cancer Treatment”
Megan Lynn Cavanaugh , L. James Lee , Robert J. Lee , Yasuro Sugimoto and Robert W. Brueggemeier, 2008 Annual Meeting of American Institute of Chemical Engineers November 16-21, 2008, Loews Philadelphia Hotel, Philadelphia Marriott Downtown & Pennsylvania Convention Center Philadelphia, PA

Increased expression of certain genes is common in many diseases and inhibiting these over-expressed genes is an option for treating them. RNA interference is one such gene silencing technique and is a potentially very powerful approach to cancer therapy. This collaborative research effort seeks to develop a novel liposomal vector to successfully
deliver siRNA that will suppress aromatase activity in tumor cells for breast cancer treatment. Aromatase is an enzyme that produces estrogens and the expression and activity of aromatase are higher in breast cancer cells than in normal breast tissue. Approximately 70% of breast cancers are estrogen receptor positive and the current first line treatment for postmenopausal women with these cancers is aromatase inhibitors. However, current aromatase inhibitors bring about estrogen deprivation in the entire body, causing some side-effects. Using siRNA targeting aromatase mRNA specific to breast cancer cells allows estrogen levels to be decreased in a tissue specific manner leading to diminished side-effects. Additionally, a liposomal drug delivery system will not only help prevent siRNA degradation, but can increase circulation time, target tumor cells, improve cellular uptake, and lower the required effective dose.

II. "Selective Regulation of Aromatase Expression for Drug Discovery" Robert W. Brueggemeier, Bin Su, Michael V. Darby, and Yasuro Sugimoto, 2008 International Aromatase Conference, October 13 to 17, 2008 Shanghai, China

Abstract for the 2008 International Aromatase Conference:

Selective Regulation of Aromatase Expression for Drug Discovery.

Robert W. Brueggemeier, Bin Su, Michael V. Darby, and Yasuro Sugimoto,
College of Pharmacy and OSU Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, U.S.A.

Aromatase is a particularly attractive drug target in the treatment of hormone-responsive breast cancer, and aromatase activity in breast cancer patients is greater in or near the tumor tissue compared with the normal breast tissue. Complex regulation of aromatase expression in human tissues involves alternative promoter sites that provide tissue-specific control. Previous studies in our laboratories suggested a strong association between aromatase (*CYP19*) gene expression and the expression of cyclooxygenase (*COX*) genes. Additionally, COX selective inhibitors can suppress CYP19 gene expression and decrease aromatase activity. Our current hypothesis is that pharmacological regulation of aromatase can act locally to decrease the biosynthesis of estrogen and may provide additional therapy options for patients with hormone-dependent breast cancer. Two pharmacological approaches are being developed, one approach utilizing small molecule drug design and the second approach involving mRNA silencing technology. The small molecule drug design approach focuses on the synthesis and biological evaluation of a novel series of sulfonanilide analogs derived from COX-2 selective inhibitors. Combinatorial chemistry approaches were used to generate diversely substituted novel sulfonanilides by parallel synthesis. The compounds suppress aromatase enzyme activity in SK-BR-3 breast cancer cells in a dose and time dependent manner, and structure activity analysis does not find a correlation between aromatase suppression and COX inhibition. Real-time PCR analysis demonstrates that the sulfonanilide analogs decrease aromatase gene transcription in breast cells. Furthermore, the sulfonanilide compounds selectively decrease aromatase gene expression in breast cancer cells, without exhibiting cytotoxic or apoptotic effects at low micromole concentrations. A ligand based pharmacophore model for selective aromatase modulation (SAM) identified four key chemical features: one aromatic ring, two hydrogen bond acceptors, and one hydrophobic function. In the second approach, short interfering RNAs (siRNA) were designed targeting human aromatase mRNA. Treatment of breast cancer cells with siRNAs targeting aromatase (siAROMs) completely masked the aromatase enzyme activity and resulted in suppression of CYP19 mRNA. Thus, these results suggest that the novel sulfonanilides and the siRNAs targeting aromatase expression may be valuable tools for selective regulation of aromatase in breast cancer.

III. “Liposomal siRNA Delivery to Silence Aromatase” Yasuro Sugimoto, Megan Cavanaugh, L. James Lee, Robert J. Lee and Robert W. Brueggemeier, 2008 International Aromatase Conference, October 13 to 17, 2008 Shanghai, China

Abstract for the 2008 International Aromatase Conference:

Liposomal siRNA Delivery to Silence Aromatase

Yasuro Sugimoto, Megan Cavanaugh, L. James Lee, Robert J. Lee and Robert W. Brueggemeier
Colleges of Pharmacy and Engineering, NSF Nanoscale Science and Engineering Center, and OSU Comprehensive Cancer Center, The Ohio State University, Columbus Ohio 43210, U.S.A.

Approximately 70% of breast cancers are ER positive and adjuvant antiestrogen therapy is considered as a primary therapy for those cancer patients. Local estrogen production can regulate the cancer progression, particularly in postmenopausal women with breast cancer. Current aromatase inhibitors (AIs) demonstrate global estrogen deprivation, which can result in some potential side effects. An AI specifically targeting breast cancer and its surrounding adipose tissue may lead to more effective therapies with diminished side effects. Furthermore, the combination of a novel RNAi therapeutic approach with a novel lipid nanoparticle (LN) drug delivery system provides particle stabilization, colloidal stability, steric stabilization, cellular internalization and endosome release, prolonged circulation, and tumor cell targeting.

Our hypothesis is that an siRNA specific to aromatase mRNA allows estrogen levels to be decreased in breast cancer cells leading to diminished side effects. We have designed and synthesized an siRNA library specific for the human aromatase. The siRNAs were evaluated in intact breast cancer cell lines, breast cancer cells transfected with human aromatase cDNA, and choriocarcinoma cell lines. The expression levels of mRNA and aromatase activities were measured to examine the silencing effect 48 hours after siRNA transfection. We have identified the optimum siRNA sequence to silence the aromatase in all cell lines evaluated. We also developed a novel liposomal vector to successfully deliver siRNA. The siRNA combined with DOTAP/Chol liposomes resulted in a consistent particular size of 50 – 100 nm. More importantly, the transfection of the complex lead to aromatase activity silencing in the breast cancer cell line and a stably transfected cancer cell line with aromatase.

Thus, these results suggest that the novel siRNAs-LN targeting aromatase expression may be valuable tools for selective regulation of aromatase in breast cancer.

IV. “Liposomal siRNA delivery to silence aromatase” Yasuro Sugimoto, Megan L. Cavanaugh, Longzhu Piao, Robert J. Lee, L. James Lee, Robert W. Brueggemeier. AACR 100th Annual Meeting 2009, April 18-22, 2009 in Denver, CO

Approximately 70% of breast cancers are ER positive and adjuvant antiestrogen therapy is considered as a primary therapy for those cancer patients. Local estrogen production can regulate the cancer progression, particularly in postmenopausal women with breast cancer. Current aromatase inhibitors (AIs) demonstrate global estrogen deprivation, which can result in some potential side effects. An AI specifically targeting breast cancer and its surrounding adipose tissue may lead to more effective therapies
with diminished side effects. Furthermore, the combination of a novel RNAi therapeutic approach with a novel lipid nanoparticle (LN) drug delivery system provides particle stabilization, colloidal stability, steric stabilization, cellular internalization and endosome release, prolonged circulation, and tumor cell targeting. Our hypothesis is that an siRNA specific to aromatase mRNA allows estrogen levels to be decreased in breast cancer cells leading to diminished side effects. We have designed and synthesized an siRNA library specific for the human aromatase. The siRNAs were evaluated in intact breast cancer cell lines, breast cancer cells transfected with human aromatase cDNA, and choriocarcinoma cell lines. The expression levels of mRNA and aromatase activities were measured to examine the silencing effect
48 hours after siRNA transfection. We have identified the optimum siRNA sequence to silence the aromatase in all cell lines evaluated. We also developed a novel liposomal vector to successfully deliver siRNA. The siRNA combined with DOTAP/Chol liposomes resulted in a consistent particular size of
70 - 80 nm. More importantly, the transfection of the complex lead to aromatase activity silencing in the breast cancer cell line and a stably transfected cancer cell line with aromatase. Thus, these results suggest that the novel siRNAs-LN targeting aromatase expression may be valuable tools for selective regulation of aromatase in breast cancer. (Supported by NCI R01 CA73698, NSF IGERT No. 0221678, DoD W81XWH-08-1-0521, W81XWH-08-1-0610)

V. “siRNA delivery and silencing by cationic liposomal nano-particles in breast cancers”, Longzhu Piao, Hong Li, Yasuro Sugimoto, Robert W. Brueggemeier, Robert J Lee, 2009 AAPS Annual Meeting and Exposition 11/7/2009 - 11/12/2009, Los Angeles, CA

Purpose: RNA interference (RNAi)-molecules are potent effectors of post-transcriptional gene silencing. The purpose of present study was to develop new cationic liposomal formulation for delivery of siRNA to breast cancer. Therefore, GFP siRNA was used to evaluate gene silencing efficiency. In vivo anti-cancer efficacy of cationic liposomal formulation was also evaluated by using RAN siRNA.
Methods: Cationic liposomal formulation with GFP-siRNA (GFP-siRNA-Lip) was prepared by ethanol injection method and characterized by particle size and zeta potential. GFP over-expressed MCF- 7, SK-BR-3 and MDA-MB-231 cells were treated with GFP-siRNA-Lip and GFP gene down-regulation was determined by real-time PCR. Fluorescent density of GFP over-expressed cells by treatment of GFP-siRNA-Lip was determined fluorescence intensity by a plate reader (ex: 485 nm, em: 535 nm) and flow cytometry. In vivo anti-cancer efficacy was evaluated by using KB cell inoculated DBA/2 mice.
Results: GFP-siRNA-Lip was successfully prepared by ethanol injection method. The particle size of GFP-siRNA-Lip was 67.8±3.5 nm and zeta potential was +22.1 mV. In GFP overexpressed MCF-7 cells, GFP-siRNA-Lip were significantly down-regulated GFP gene expression compared with control and scramble siRNA contained cationic liposomes. The down-regulation efficiency of GFP-siRNA-Lip was better than lipofectamine. Similar results were observed in the GFP overexpressed SK-BR-3 and MDA-MB-231 cells. Fluorescent intensity of GFP/MCF-7 cells was decreased 60.5% by treatment of GFP-siRNA-Lip. In addition, flow cytometry data also showed significant GFP gene down-regulation in GFP overexpressed MCF-7, SK-BR-3 and MDA-MB-231 cells.
Conclusions: Theses results suggest that our new cationic formulation as a non-viral carrier could be used to siRAN delivery of breast cancer.
Keywords: RNA interference (RNAi); Gene silencing; Cationic lipid; Nano-particle;
