Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Thomas Jefferson University

2. **Reporting Period (start and end date of grant award period):** 01/01/2010 – 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Joy Soleiman, MPA

4. **Grant Contact Person's Telephone Number:** 215-955-5684

5. **Grant SAP Number:** 4100050910

6. **Project Number and Title of Research Project:** 3 - The Role of Lipogenesis Pathway in Endometrial Cancer Progression

7. **Start and End Date of Research Project:** 01/01/2010 – 06/30/2012

8. **Name of Principal Investigator for the Research Project:** Chenguang Wang, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 393,699.66

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Wang, Chenguang	Principal Investigator	3% Yr1, 13%Yr2	28,366
Zhou, Jie	Instructor	100% Yr.1	56,754
Gu, Weiting	Visiting Scholar	100% Yr.1 & 2	32,669
Li, Wiehua	Visiting Scholar	100% Yr.1 & 2	24,977
DiNatale, Gabriel	Graduate Student	100% Yr.2	3,798
Lin, Liz	Graduate Student	100% Yr. 2	3,339

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
NONE			

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
NONE		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No _____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes ____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the

application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Molecular mechanisms governing enhanced lipogenic signaling in breast cancer	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$1,250,000	$0
Characterization of a Novel Lipogenesis Inhibitor to Treat Cancer	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$155,000	$0

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes __X____ No_____

If yes, please describe your plans:

We applied for RO1 and RO3 grants, both applications received scores but were not funded. We plan to resubmit the revised applications in 2014. My lab was also working collaboratively with investigators from Thomas Jefferson University and other regional institutes.

12. Future of Research Project. What are the future plans for this research project?

We have recently shown that SREBP1, the master regulators of lipogenesis in diverse organisms, is overexpressed in endometrial cancer (EC). Importantly, we have established that SREBP1 is a therapeutic target for cancer intervention by showing that the knockdown of SREBP1 impairs tumor growth in vivo. A small library of boron-containing compounds was developed using Limited Rational Design Approach and compounds in the library were screened using *FASN* gene promoter-driven luciferase reporter as a surrogate measure of SREBP activity. BF175 and BF102 were identified as the most active molecules to inhibit lipogenic signaling. Through collaboration with Dr. Das, the inventor of this library, we

found that BF175 in food significantly reduced the fat levels in Drosophila and mice models. Our preliminary data in cell culture also showed that BF175 inhibits SREBP1-depdendent gene expression, lipogenesis, and cell proliferation. In the future we will continue to validate the inhibitory effects of BF175 on tumor growth using a variety of cell culture and in vivo models and to explore the underlying mechanisms leading to the inhibition of SREBP1 activation.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes ____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	1			
Female	1	1	1	1
Unknown				
Total	**2**	**1**	**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	1			
Non-Hispanic	1	1	1	1
Unknown				
Total	**2**	**1**	**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian	1	1	1	1
Other				
Unknown				
Total	**2**	**1**	**1**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No _____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes ____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This research project allowed us to develop a small molecule inhibitor of tumor growth. We established a new method and built new experimental tools. This project also allowed us to train students.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes ___X_____ No_____

If yes, please describe the collaborations:

1. Collaborated with Dr. Nianli Sang at Drexel University, we published three papers together.
2. Collaborated with Bin Chen at the University of the Sciences at Philadelphia. The collaboration led to two publications and one grant submission.
3. Collaborated with Dr. Junyuan Ji at Texas A&M Health Science Center. We have published three papers through this collaboration and submitted one grant application to NIH.

16(B) Did the research project result in commercial development of any research products?

Yes ____X_____ No_____

If yes, please describe commercial development activities that resulted from the research project:

We have submitted one patent application for the small molecule inhibitor of lipogenesis. The patent has been licensed for further development.

16(C) Did the research lead to new involvement with the community?

Yes_____ No _____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

PROJECT OVERVIEW

The significant increase of lipogenesis is a metabolic hallmark of rapidly proliferating tumor cells. Although most normal cells acquire the bulk of their fatty acids from circulation, tumor cells synthesize more than 90% of required lipids de novo. Consistent with a great demand for lipid synthesis, diverse human cancer cells express high levels of lipogenic enzymes, such as fatty acid synthase (FASN). Although the clinical correlations between the over-expression of lipogenic enzymes and tumorigenesis are well documented, little is known about the molecular

mechanisms by which the lipogenic pathway is enhanced in tumorigenesis. The sterol regulatory element-binding protein 1 (SREBP1) and peroxisome proliferator-activated receptor gamma (PPARgamma) are master regulators of lipogenesis in diverse organisms.

Previous studies have established that FASN, one of the major transcriptional targets of SREBP1, promotes synthesis of various fatty acids, which then serve as ligands for PPARgamma activation. However, whether SREBP1, FASN, and PPARgamma contribute to endometrial carcinogenesis has not been systematically investigated. Excitingly, the preliminary data of this project suggests that DACH1, a newly identified transcriptional repressor, may negatively regulate FASN expression. DACH1 is down-regulated in endometrial cancers, and the re-introduction of DACH1 reverts tumor-associated phenotypes. In this project, we hypothesize that increased SREBP1 and loss of DACH1 are associated with endometrial cancer progression. They may function collaboratively to promote endometrial tumorigenesis through enhanced lipogenesis.

To test this hypothesis, the correlation between cancer progression and lipogenic gene expression in human endometrial cancer samples will be determined. By using three independent endometrial tissue microarrays and frozen tissues together with experimental approaches including immunohistochemistry (IHC) staining, quantitative real-time PCR (qRT-PCR), and Western Blot, determination will be made of the correlation between 1) endometrial cancer (EC) progression and levels of the key lipogenic enzymes, such as SREBP1, FASN, and PPARgamma; 2) lipogenic gene expression and other known prognostic factors; and 3) evaluation of the diagnostic values of SREBP1 in predicting survival and metastasis in EC.

Expected Research Outcomes and Benefits

Several reports have noted an enhanced lipogenic pathway in advanced stages of human cancer compared to normal tissue, including human endometrial cancer, in which the PPARgamma and SREBP1 targeted genes such as FASN and SCD1 are induced to high levels of expression. Interestingly, elevated expression of SREBP1 coincided with malignant transformation, cancer progression, and metastasis for several cancer types, particularly hormone-responsive tissues including breast and prostate cancers. The role of SREBP1 in endometrial cancer is largely unknown. Given the evidence of elevated expression of SREBP1 transcriptional target genes, one aim is to first determine the SREBP1 expression status in endometrial cancer. Increased nuclear staining for SREBP1 in higher grade (G2-G3) tumors from our pilot study suggests that enhanced SREBP1 transactivation may contribute to endometrial cancer progression through induction of lipogenic gene expression and lipogenesis.

In this project, there will be expansion of analysis to determine whether increased expression and/or nuclear translocation of SREBP1 are the preferred mechanism for lipogenesis in human endometrial cancer. This project will allow identification of the molecular mechanisms underlying lipogenesis-dependent tumor growth in endometrial cancer. This is important because the data will provide new strategies for targeting endometrial cancer progression; according to this model, targeted SREBP1 inhibition may efficiently inhibit tumor growth. Furthermore, together with known prognostic factors, SREBP1, DACH1, and PPARgamma may serve as diagnostic biomarkers for human endometrial cancer and may be used to predict response to

targeted therapies.

Research Background

The number of women with newly diagnosed endometrial cancer (EC) has increased 20% since 1987, yet the number of deaths from EC posted a 168% increase during the same time period. Estimated new cases and deaths from EC in the United States in 2011 are 46,470 and 8,120, respectively (http://www.cancer.gov/cancertopics/types/endometrial). Although well-differentiated endometrial cancers have a favorable outcome, the poorly differentiated form has a worse prognosis, largely due to lack of biomarkers and therapeutic targets to predict and to restrict cancer progression. Therefore, identifying new bio-markers and therapeutic targets may provide novel approaches that can improve the clinical outcome of endometrial cancer.

Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that regulate lipid homeostasis by controlling the expression of the key and rate-limiting enzymes required for cholesterol and fatty acid (FA) synthesis. In mammals, SREBPs are composed of three isoforms, SREBP1a, SREBP1c and SRBEP2: SREBP1a and SREBP1c, encoded by a single gene SREBF1 with alternative promoter usage, mainly control lipogenic gene expression, while SREBP2, encoded by a separate gene SREBF2, predominantly regulates cholesterogenic gene expression [1, 2]. As transcription factors, SREBPs are unique in that they are synthesized as inactive precursors bound to the endoplasmic reticulum (ER) membranes and their processing is mainly controlled by the intracellular sterol content. When sterol level decreases, the SREBP precursors are transported to Golgi apparatus, where they undergo a sequential two-step cleavage process. As a result, the NH2-terminal active domain, also known as the mature SREBP, is released and translocated into the nucleus (designated nSREBP), which then activates SREBP target genes to increase the biosynthesis of cholesterol and FAs. In contrast, when the intracellular sterol level is high, the SREBP precursors will remain in the ER. This model provides an elegant explanation of how intracellular sterol and FA homeostasis is maintained [3-9]. The unique regulation and activation properties of each SREBP isoform facilitate the co-ordinated regulation of lipid metabolism.

Lipogenesis is almost universally upregulated in human cancers [10]. Consistent to an essential role of SREBP1 in sensing and regulating intracellular lipid homeostasis, increased expression of SREBP1 has been detected in colorectal carcinoma, breast and prostate cancer, and hepatocarcinoma (11-14). Moreover, elevated expression of SREBP1 is closely correlated with malignant transformation, cancer progression, and metastasis for several cancer types, particularly hormone responsive tissue-derived cancers, such as breast and prostate cancers [11, 13, 15, 16]. For examples, SREBP1 expression correlates with FASN and Ki-67 expression in colorectal cancer, indicating a role for SREBP1 in supporting rapid cellular proliferation [16]. SREBP1 is elevated in clinical prostate cancer samples compared to benign prostatic hypertrophy [12]. Gene expression profiling of hepatocellular carcinoma (HCC) tissue and non-cancerous liver tissue showed increased lipogenic signaling in HCC. ElevatedSREBP1 expression in hepatocellular carcinoma is a known predictor of increased mortality [13, 15]. Overexpression of SREBP1 in human hepatoma HuH7 and Hep3B cells enhanced cellular proliferation and foci formation, while knockdown of SREBP1 in these cells reduced cell replication and anchorage-independent cell growth [15]. A dramatic increase of SREBP1 has been

correlated with the progression of prostate cancer towards androgen-independence [12]. Oncogenic transformation of normal breast epithelial cells was accompanied by increased SREBP1 and FASN expression, consistent with the observation of increased SREBP1 abundance in human breast cancers [17-19]. Previous studies have established that SREBP1, through induction of FASN and subsequent fatty acids production, regulates PPARγ transactivation [20, 21].

Although lipogenesis is known to be elevated in EC cells, the role of SREBP1 in endometrial cancer remains largely unclear. Herein, we report that the nuclear SREBP1 is significantly increased in endometrial cancer and the expression levels are positively correlated to cancer progression. Importantly, knockdown of endogenous SREBP1 not only reduced SREBP1 target gene expression, but also suppressed cellular proliferation and colony formation of endometrial cancer cells. Furthermore, xenograft tumor model clearly demonstrated that reduction of SREBP1 expression reduced tumor growth rate. These observations indicate that SREBP1 is required for endometrial cancer cell proliferation and cancer progression, supporting the model that enhanced SREBP1 transactivation may contribute to endometrial cancer progression through induction of lipogenic gene expression and lipogenesis.

Materials and Methods

Endometrial cancer specimens and Immunohistochemistry (IHC) staining

Formalin-fixed and paraffin-embedded tumor specimens used in this study were from commercial source (Creative Biolabs, Shirley, NY) and from tissue bank of the 90th Hospital of Jinan, China. All tumors were primary and untreated before surgery with complete clinicopathological information. Tumor size was defined as the maximum tumor diameter measured on the tumor specimens at the time of operation. H&E-stained sections of specimens were reviewed and the diagnosis confirmed by an expert gynecologic pathologist. All of the specimens were anonymous and tissues collected in compliance with institutional review board regulations. Patients with endometrial cancer and tumor characteristics for this study population are is summarized in Table 1. Endometrial cancer tissues from the 90th Hospital of Jinan were built into a 60-core array with 2 mm diameter of the core size. Adjacent normal tissues were included for some cancer tissues. Endometrial Cancer cDNA Arrays (TissueScan™ cDNA Arrays) were purchased from OriGene Technologies, Inc. (Rockville, MD).

IHC staining for SREBP1 was performed on the paraffin-embedded tissue blocks in the Kimmel Cancer Center Pathology Core Facility at Thomas Jefferson University. Hematoxylin and eosin (H&E) staining were reviewed to ensure the cancer tissue and normal epitheliums. IHC staining for these markers was performed on 5-μm thick section. Briefly, tissue slides were de-paraffinized with xylene and rehydrated through a graded alcohol series. The endogenous peroxidase activity was blocked by incubation in a 3% hydrogen peroxide solution for 15 minutes. Antigen retrieval was carried out by immersing the slides in 10 mM sodium citrate buffer (pH 6.0) and maintained at a sub-boiling temperature for 5 minutes. The slides were rinsed in phosphate-buffered saline and incubated with 10% normal serum to block non-specific staining. The slides were then incubated with the primary antibody (anti-SREBP1, K-10, Santa Cruz) overnight at 4°C in a humidified chamber.

All of staining was assessed by pathologists blinded to the origination of the samples using a

semi-quantitative method. Each specimen was assigned a score according to the intensity of the nucleic and cytoplasmic staining. Tissue was scored (H-score) based on the total percentage of positive cells and the intensity of the staining (1+, 2+, or 3+), where H = (%"1+" x 1) + (%"2+" x 2) + (%"3+" x 3). A minimum of 100 cells were evaluated in calculating the H-score.

Statistical Analysis

Means of continuous variables for SERBP-1 staining intensity between endometrial cancer and each normal endometrial phase or within normal endometrial phases were compared by one-way analysis of variance (multiple comparisons). The comparison between the clinicopathologic characteristics of endometrial cancer and SERBP-1 staining intensity was evaluated with the Mann-Whitney U test. All statistical tests were two-sided, and P values less than 0.05 were considered as statistically significant. The statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL).

Plasmids, antibodies, and reagents

A set of five of shRNAs targeting human SREBF1 were purchased from Open Biosystems (Catalog number: RHS4533, Lafayette, CO). The 1000-bp promoter of FASN was amplified from genomic DNA of AN3 CA cells and subcloned into Kpn I/Bgl II of pGL3-basic vector (Promega). Mouse Scd1 promoter-driven luciferase reporter was a gift from Dr. Ntambi [(22)]. pcDNA4-FLAG-SREBP1a was obtained from Addgene [(23)]. All the mutant constructs for SREBF1 promoter-driven luciferase reporter were kindly provided by Dr. Yamada [(24)]. The retroviral vector (MSCV-IRES-GFP) was described in our prior publication [(25)]. Anti-SREBP1 (K-10 and H-160), anti-FASN (H300), anti-actin (C4) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA),anti-SCD (MC38) and anti-LC3A/B antibodies were purchased from Cell Signaling Technology (Danvers, MA) and anti-ACLY antibody was from Abcam (Cat.# ab40993, Cambridge, MA).

Cell Culture

The human embryonic kidney 293T cells (HEK 293T) were maintained in DMEM containing 1% penicillin/streptomycin and supplemented with 10% fetal bovine serum (FBS). Endometrial cancer cell lines including ECC-1, HEC-1A, RL95-2, KLE, and AN3 CA were purchased from the American Type Culture Collection (ATCC). The basal culture medium are: RPMI-1640 (ECC-1), McCoy's 5a (HEC-1A), DMEM: F12 (RL95-2, KLE), and Eagle's Minimum Essential Medium (AN3 CA) according to ATCC. For cell maintenance, the basal medium was supplemented with 10% fetal bovine serum (FBS) except ECC-1 with 5% FBS. Under lipid-free culture condition as indicated, the basal medium was supplemented with 5% lipid-depleted FBS purchased from Cocalico Biologicals (Catalogue number 55-0116, Reamstown, PA).

Cell transfection and transduction

For transient transfection, Superfect Transfection Reagent (Qiagen, Valencia, CA) was used following manufacturer’s protocol. For cell transduction, lenti-viruses were prepared using Trans-Lentiviral shRNA Packaging Kit following manufacturer’s instruction (Open Biosystems) with modifications. Briefly, lenti-viral vector expressing shRNA will be introduced into HEK 293T cells by transient co-transfection with helper virus with calcium phosphate precipitation. After 6 hrs., cell culture medium was replaced and cells were allowed to grow for 36 hrs. to produce viruses. The supernatant was then collected and filtered through a 0.45-μm filter. Cells

were infected at approximately 70% confluence in DMEM supplemented with 8 μg/ml of polybrene. The following day the medium was changed to basal medium supplemented with 10% FBS and cultured for further assays. Cells were stably selected by supplementing the medium with 2 μg/ml puromycin for two weeks. The knockdown efficiency was determined by either Western blot or qRT-PCR assays.

RNA isolation and quantitative RT-PCR

Total RNA was prepared using TRIzol Reagent (Invitrogen, Carlsbad, CA) following manufacturer's instructions. 5 μg of total RNA was subjected to reverse transcription to synthesize cDNA using the SuperScriptTM II Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA). For qRT-PCR, each reaction (25 μl) consisted 1 μl reverse transcription cDNA product and 100 nM of each primer. The primers used for qRT-PCR are listed as below:

Gene symbol	Forward primer sequence	Reverse primer sequence	Amplicon size
SREBP1a	5'-CGGCGCTGCTGACCGACATC	5'-CCCTGCCCCACTCCCAGCAT	104bp
SREBP1c	5'-GCGCAGATCGCGGAGCCAT	5'-CCCTGCCCCACTCCCAGCAT	116bp
SREBP2	5'-CAAGCTTCTAAAGGGCATCG	5'-AGTAGGGAGAGAAGCCAGCC	140bp
FANS	5'-CACAGGGACAACCTGGAGTT	5'-ACTCCACAGGTGGGAACAAG	97bp
SCD1	5'-CGACGTGGCTTTTTCTTCTC	5'-CCTTCTCTTTGACAGCTGGG	70bp
ACLY	5'-GCCCATCCCCAACCAGCCAC	5'-TTGCAGGCGCCACCTCATCG	137bp
ADIPOQ	5'-TCCTGCCAGTAACAGGGAAG	5'-AGGGGAAGTGTCAGTACCCG	168bp
aP2	5'-CTCTCCGTTCAGATTGAAGGGG	5'-AATCCCGCCTCCATCCTAACT	122bp
GAPDH	5'-GAGTCAACGGATTTGGTCGT	5'-TTGAGGTCAATGAAGGGGTC	103bp

Cellular proliferation and apoptosis assays

For cell proliferation assays, cells were stably transfected with shRNA targeting SREBF1 and control were seeded at a density of 2 x 105 cells per well in 6-well culture device in DMEM containing 10% FBS. The total number of cells was counted for 5 days. Data are from at least three experiments done in triplicate (mean ± SEM). Cell death was determined by PE Annexin V Apoptosis Detection Kit (BD Biosciences) following manufacturer's instructions.

Colony formation assays

A total of 2,000 cells were seeded in 100-mm plates and allowed to grow for two weeks. The culture medium was replaced every three days with fresh DMEM supplemented with either regular FBS or lipid-depleted FBS. The number of colonies formed per plate was stained with crystal violet and determined quantitatively by Gel-Pro Analyzer (Media Cybernetics, Inc. MD).

Subcutaneous Tumor Implantation

AN3 CA cells (2 x 106) with either knockdown of SREBP1 or vector control were implanted by subcutaneous injection in the flank of male 6- to 8- week old SCID mice. Comparisons were made for 10 animals in each group between AN3 CA/vector, AN3 CA/shSREBF1#2, and AN3 CA/shSREBF1#4. The tumor growth rates were examined using serial caliper measurements. The tumor volume were calculated using the equation (a x b2)/2 where "a" and "b" are length and width of the tumor, respectively. At the completion of the experiments, tumors were excised, weighed and statistical significance of differences in tumor volume were logarithm-transformed and analyzed using a linear mixed model. Separate slope and intercepts were computed for each

group (Vector, #2, #4), then we compared across groups using a global test followed by pair-wise comparisons via linear contrasts. Data prior to Day 12 were ignored due to zeroes at Day 0 (inability to take logarithms) and an initial non-linearity /change in some of the animals growth patterns prior to Day 12. Thus, the intercept at Day 12 is interpretable as initiation of growth and the slope is interpretable as rate of growth. For the models, we report coefficients, confidence intervals of coefficients and 2-sided p-values.

SUMMARY OF RESEARCH DATA

Elevated expression of SREBP1 in endometrial carcinoma compared to the adjacent normal endometrium

It has been reported that FASN, the lipogenic gene encoding fatty acid synthase, is overexpressed in endometrial cancers [26, 27]; however, the underlying mechanisms are still unknown [28]. SREBP1 is the major transcription factor that directly activates the expression of FASN and other lipogenic genes [21, 29]. Therefore, we asked whether increased SREBP1 expression and/or activity contribute to the elevated FASN expression in EC. To determine the levels of SREBP1 expression, we performed immunohistochemical staining on formalin-fixed, paraffin-embedded sections using anti-SREBP1 antibody. The levels of nuclear and cytoplasmic SREBP1 were scored for both mature and precursor forms, respectively. As shown in figure 1, SREBP1 is expressed in both normal and cancerous tissues, but its levels were significantly increased in poorly-differentiated EC. The majority of SREBP1 was found in the cytoplasm of epithelial cells of normal endometrium and well-differentiated tumors, while nuclear SREBP1 was mainly detected in high-grade tumors including moderately differentiated to poorly differentiated (Fig. 1). These observations indicate that SREBP1 expression and activation positively correlates with the progression of endometrial cancer.

SREBP1 status in endometrium during menstrual cycle and post-menopause

Because endometrial cancer progression correlates with the menopausal status, we characterized the expression of SREBP1 in endometrium during menstrual cycle and in post-menopausal endometrium. As a result of cyclic steroid hormone levels throughout menstrual cycle, the endometrium undergoes characteristic proliferative, secretory, and menstrual phases, and displays a wide spectrum of normal and pathological appearances. As shown in figure 2, SREBP1 protein was markedly increased in glandular epithelial cells during proliferative phase, and SREBP1 protein was located in both nucleus and cytoplasm. In contrast, at the secretory phase, the epithelial cells in endometrium lose the expression of SREBP1 protein, while the nuclear distribution of SREBP1 became negligible (Fig. 2). The observation of increased SREBP1 expression and activation (nuclear translocation) in proliferative phase is consistent with the notion that the proliferating cells in endometrium require de novo lipogenesis. SREBP1 protein was not detectable in the majority of post-menopausal endometrium, as well as the stromal cells regardless the endometrial phases (Fig. 2 or data not shown).

Enhanced SREBP1 expression and nuclear translocation in atypical hyperplasia

Atypical endometrial hyperplasia (AH) is a precancerous condition. Patients with AH have increased risk in developing invasive endometrial cancer comparing to those patients who have hyperplasia without atypia. To test whether the expression and distribution of SREBP1 correlates with the AH status in endometrial hyperplasia, we performed immunohistostaining of SREBP1 in hyperplasic endometrial samples. As shown in figure 3, we observed significant differences in

both the expression levels and the distribution of SREBP1 in hyperplasic tissues without atypia compared to normal tissues. SREBP1 expression in atypical hyperplasia, when scored at whole cell level, was significantly higher than that in normal and non-atypical hyperplasia (Fig. 3). In addition, cytoplasmic SREBP1 was increased in AH when compared to normal tissue (Fig. 3). Elevated nuclear SREBP1 in AH was observed, but did not reach the significant levels when compared to normal ($p = 0.180$), non-AH ($p = 0.173$), and EC ($p = 0.958$), which could be due to the limited number of patient samples. These results indicate that SREBP1 activation occurs in AH, the precancerous condition, which may contribute to cancer progression. SREBP1 is responsible for lipogenic gene expression in endometrial cancer cells.

The levels of SREBP1 can be regulated by multiple mechanisms including transcriptional or post-translational regulation [30, 31]. To examine whether increased SREBP1 protein in EC patient samples was due to enhanced gene expression, we performed quantitative RT-PCR (qRT-PCR) analysis on a panel of cDNA samples prepared from surgically resected samples of normal and cancerous tissues and compared the mRNA levels of SREBF1a, SREBF1c, SREBF2, and SCD (Stearoyl CoA desaturase), a transcriptional target of SREBPs. As shown in figure3A, SREBP1c expression was significantly increased; while the levels of SREBP1a and SREBP2 transcripts are markedly reduced in cancer tissues. Similar to SREBF1c, the mRNA levels of SCD were significantly increased in cancer, which is consistent to increased SREBP1 protein expression and activation as evidenced by nuclear translocation.

Having established the correlation between SREBP1 expression and endometrial cancer progression (Fig. 1 and Fig. 2), we next determined whether elevated levels of SREBP1 was required for expression of the lipogenic genes in EC cells. To answer this question, we analyzed SREBP1 expression in five commonly used endometrial cancer cells lines, including ECC, HEC-1-A, RL95-2, KLE, and AN3 CA. As shown in figure 3B, SREBP1 expression was undetectable in the well-differentiated ECC-1 cells, but it was highly expressed in medium and poorly differentiated RL95-2 and AN3 CA cells. Since the SREBP1 antibody cannot distinguish SREBP1a from SREBP1c proteins, we performed qRT-PCR to determine which SREBP1 isoform was predominantly expressed. RL95-2 cells express both isoforms at relatively high levels; in contrast, AN3 CA expresses SREBP1a, but not SREBP1c (data not shown). Next, we determined the expression of SREBP target genes, such as FASN, SCD, and ACLY (ATP citrate lyase). Relatively high expression of FASN, SCD1, and ACLY were detected in AN3 CA cells (data not shown). To test whether the endogenous SREBP1 was required for lipogenic gene expression, we chose AN3 CA cells and depleted SREBP1 by using a shRNA approach. We used five different shRNAs targeting mRNA ofSREBF1. As shown in figure 3C-D, shRNA#2 and shRNA#4 exhibited high knockdown efficiency as confirmed at both protein and mRNA levels. The expression of SREBP1 target genes was also significantly reduced (Fig. 3D), supporting that the expression of these genes is at least in part dependent on SREBP1 function.

<u>SREBP1 is required for cell proliferation</u>

Cell proliferation is not only tightly controlled by mitogenic signaling, but also requires activation of biosynthetic pathways for the generation of macromolecules, including proteins and lipids. Because SREBP1 regulates lipogenesis, the metabolic process that supplies cells with phospholipids, we expected that the knockdown of endogenous SREBP1 would suppress cell proliferation and growth. To determine the functional consequences of SREBP1 inactivation in

cultured EC cells, we analyzed the effect of knockdown of SREBP1 on cell growth. As shown in figure 4A, we observed reduced cell number in shSREBF1 expressing cells compared to the vector control, suggesting the SREBP1 is required for cell proliferation. Next, to determine whether SREBP1 is required for the progression of any specific cell-cycle phases, we conducted FACs analysis. We found that depletion of SREBP1 had no significant effect on cell cycle distribution (data not shown). These observations suggest that instead of affecting certain specific cell-cycle phase, depletion SREBP1 may increase the total cell-cycle duration.
Knockdown of SREBP1 impairs EC cell colony-forming capacity and tumor growth in vivo.
To determine the role of SREBP1 in tumor colony formation, we performed the clonogenic assays. As shown in figure 4B-C, knockdown of SREBF1 by shRNA significantly reduced the number of colonies. The shRNA#4 of SREBF1 achieved nearly 100% knockdown efficiency at protein levels, which reduced the number of large colonies (size > 15) by 90%, while the shRNA#2, by reducing SREBP1 protein expression by 80% (Fig. 4C), inhibited the colony-forming capacity of AN3 CA cells by 25%. These results suggest that the colony numbers positively correlate with the levels of the SREBP1 expression. Next, we examined the contribution of SREBP1 to tumor growth in vivo by using a xenograft SCID mouse model. We implanted 2 x 106 of SREBP1 knockdown cells (shRNA#2 and shRNA#4 lines) subcutaneously, and monitored tumor growth by measuring tumor size every four days. As shown in figure 4D, E, the AN3 CA cells with depletion of SREBP1 by the shRNA#2 significantly reduced the tumor size compared to the vector control cells. Fewer tumors and slower growth rate were found in shRNA#4 AN3 CA cells implanted mice, consistent with the high efficiency of SREBP1 depletion by shRNA#4 (Fig. 4F). These observations are also in line with the observation that SREBP1 knockdown cells had decreased growth rates compared to the control cohort (Fig. 4A). Collectively, these studies demonstrate that SREBP1 is required for tumor growth in vivo.

Knockdown of SREBP1 induces apoptotic cell death

The tumor growth is resulted from the net gain of cell number, which is determined by cell proliferation and death. It has been previously shown that inhibition of SREBP1 sensitizes tumor cells to death ligand [32], suggesting that SREBP1-dependent evasion of cell death could contribute to the net cell growth. To examine whether SREBP1 is required for cell survival, we depleted SREBP1 and analyzed the sub-G1 population, which represents apoptotic cells. We observed that knockdown of SREBP1 increased the cells in sub-G1 cell fraction from 1.7% to 3.3% (data not shown), suggesting that reduction of SREBP1 leads to apoptotic cell death. Annexin V staining precedes the loss of membrane integrity of cells undergoing either apoptotic or necrotic cell death [33]. We stained the cells with Annexin V and 7-AAD. As shown in figure 5A, the early cell death marked by Annexin V-positive and 7-AAD-negative increased from 6.3% to 9% (shRNA#2) and 18.6% (shRNA#4), respectively, in cells with reduced SREBP1. Cells in late apoptosis (both Annexin V and 7-AAD positive) were low in all three cell lines, but showed an increase from 1.1% in vector control cell line to 2.1% in SREBP1 knockdown cells (#4) (Fig. 5C), which is consistent with the sub-G1 fraction of these two cell lines.

SREBP1, also known as adipocyte determination- and differentiation-dependent factor 1 (ADD1) [34], has been shown to be involved in tumorigenesis. Increased expression of SREBP1 has been reported in colorectal carcinoma, breast and prostate cancer, and hepatocarcinoma. The levels of SREBP1positively correlated with the severity of endometrial hyperplasia and cancer, which further supports the notion that SREBP1 is oncogenic. Similarly, elevated expression of

SREBP1 was reported in coinciding with malignant transformation, cancer progression, and metastasis for several cancer types, particularly hormone-responsive tissues including breast and prostate cancers [11, 13, 15, 16]. Despite these progresses, the role of SREBP1 in endometrial tumorigenesis remains unexplored.

We have shown that SREBP1 is required for the tumorigenesis of endometrial cancers. We found that SREBP1 is overexpressed in EC patients, and its activation, as indicated by nuclear translocation, correlates with high grade, poor differentiation of endometrial cancer. The lowest expression of SREBP1 was seen in post-menopausal tissues. Similar to EC tissue, atypical hyperplasic tissues exhibit an elevated SREBP1 expression. The observation of high SREBP1 expression in proliferative, hyperplasic and cancerous tissues further supports the notion that de novo lipid synthesis is enhanced in cancers. To our knowledge, this is the first report that has systematically examined the role of SREBP1 in endometrial cancer cell proliferation and tumor growth. To directly test whether SREBP1 functions as transforming oncogene, we overexpressed SREBP1 in well-differentiated ECC-1 and immortalized NIH 3T3 cells. Overexpression of SREBP1 did not show tumorigenic capability (unpublished observations), suggesting that gain of SREBP1 alone is not sufficient for tumorigenesis. Collectively, these results suggest that SREBP1 is required for promoting endometrial cancer progression rather than oncogenic transformation. We note that this observation was only made in lipid-depleted serum culture condition, where cells were completely dependent on the de novo lipogenesis to support the demand for phospholipids and cholesterol in rapid proliferating cells.

Several key enzymes involved in lipogenesis have been investigated for their potential to be targeted in cancer therapy. This rapidly growing list includes fatty acid synthase (encoded by FASN gene), acetyl-CoA carboxylase (ACACA), Stearoyl-CoA desaturase (SCD), and ATP citrate lyase (ACLY). Suppression of the protein expression and activity levels with either interfering RNA or small molecule inhibitors impairs tumor cell proliferation in cell culture and tumor growth in vivo [35-44]. In particular, targeting FASN with chemical inhibitors has been proved to be effective in repressing tumor growth [45]. However, the efficacy of the available agents in cancer therapy needs to be investigated thoroughly. As a proof of concept, our results demonstrated that SREBP1 is required for supporting tumor cell growth and survival. As a master regulator of lipogenic gene transcription, knockdown of SREBP1 efficiently reduced the expression of FASN and SCD (Fig. 3D), which may represent a novel interesting approach to block tumor progression. The present study revealed that the expression of SREBP1 was increased in endometrial carcinomas and the expression of SREBP1 protein was increased in poorly differentiated (histologically higher-grade) tumors. Importantly, knockdown of SREBP1 efficiently repressed colonigenic capacity of endometrial cancer cells and in vivo tumor growth, indicating thatSREBP1 may serve as a potential therapeutic target.

SREBP1 is synthesized as a 125-kDa precursor located in the membranes of the endoplasmic reticulum and nuclear localization is required for its activation. Besides targeting SREBP expression, strategies targeting SREBP synthesis and maturation may also provide therapeutic values. Fatostatin, a small molecule inhibitor of fatty acid synthesis by directly targeting the SREBP cleavage-activating protein (SCAP), was found to inhibit prostate cancer growth. Our current studies demonstrated the nuclear localization of SREBP1 in endometrial cancer, especially in poorly differentiated carcinoma including type II cancers (data not shown). The

underlying mechanisms through which SREBP1 is processed into matured nuclear form in endometrial cancer cells are still poorly understood and warrant further studies in the future. It is important to note that epidemiological studies have identified obesity as one of the major risk factors for endometrial cancer, where obese women have a 2~4 times greater risk of developing endometrial cancer compared to women of normal weight, regardless of menopausal status [46-50]. As population size affected by this disease is expected to grow rapidly, particularly in developing countries, endometrial cancer is expected to be a serious global health problem. This study, by establishing the role of SREBP1, the master regulator of lipogenesis, in endometrial cancer growth, may have important implications in understanding how obesity contributes to the increased risk in EC tumorigenesis.

Table 1. Clinical and pathologic characteristics of the patient cohort with endometrial cancer.

Characteristics	No. of patients	Percentage (%)
Age (y):		
≤50	28	23.14
>50	93	76.86
Menopause status		
Pre-	19	15.70
Peri-	16	13.22
Post-	86	71.08
Type of surgery		
Uterectomy	91	75.21
Exploratory curettage	30	24.79
Chemotherapy		
Yes	61	50.41
No	28	23.14
Unknown	32	26.45
Histological Grade		
I	62	51.24
II	32	26.45
III	27	22.31
Histological Type		
I	107	88.43
II	14	11.57
T Stage(patients underwent uterectomy)		
T1	79	86.81
T2	9	9.89
T3	2	2.20
T4	1	1.10
N Stage (patients underwent uterectomy)		
N0	80	87.91
N1	11	12.01
M Stage (patients underwent uterectomy)		
M0	91	100
M1	0	0
Clinical Stage		
I	72	79.12
II	6	6.59
III	12	13.19
IV	1	1.10

FIGURES

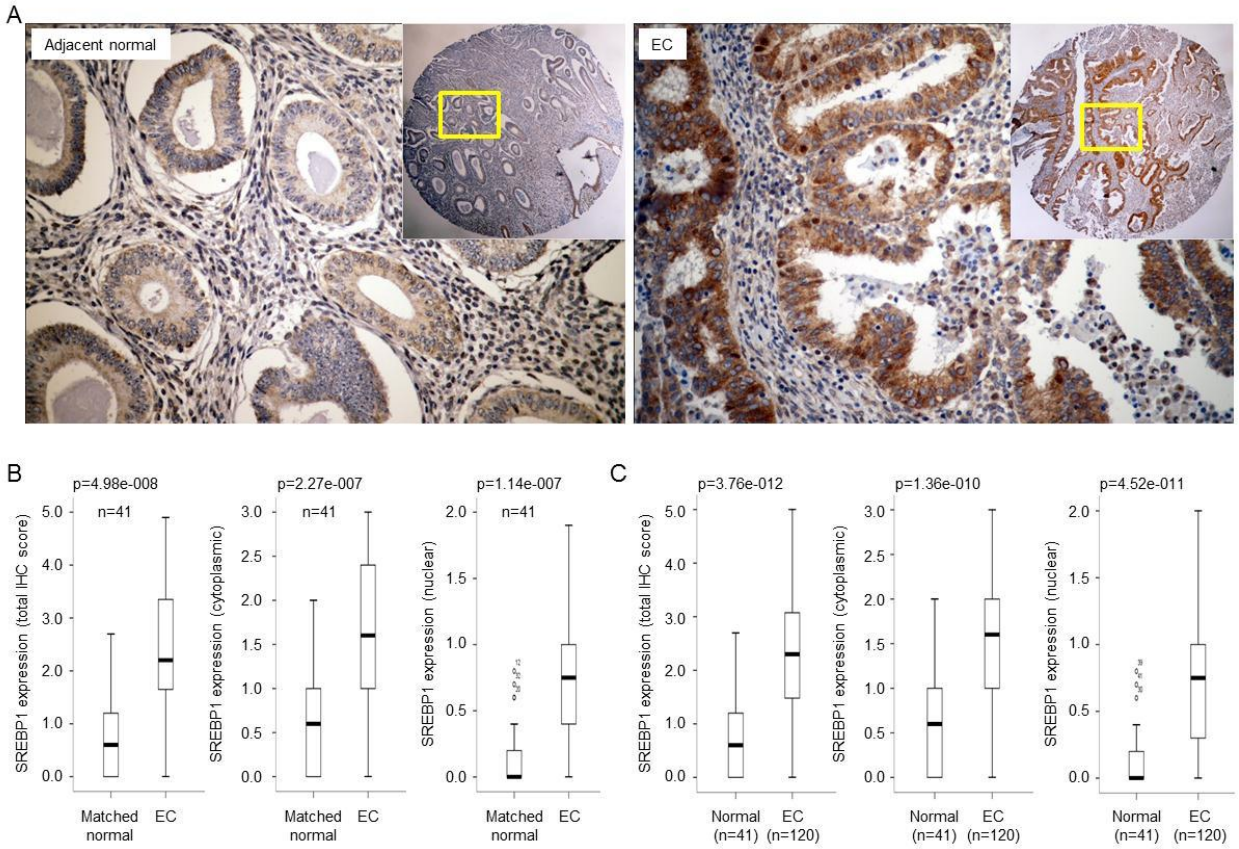
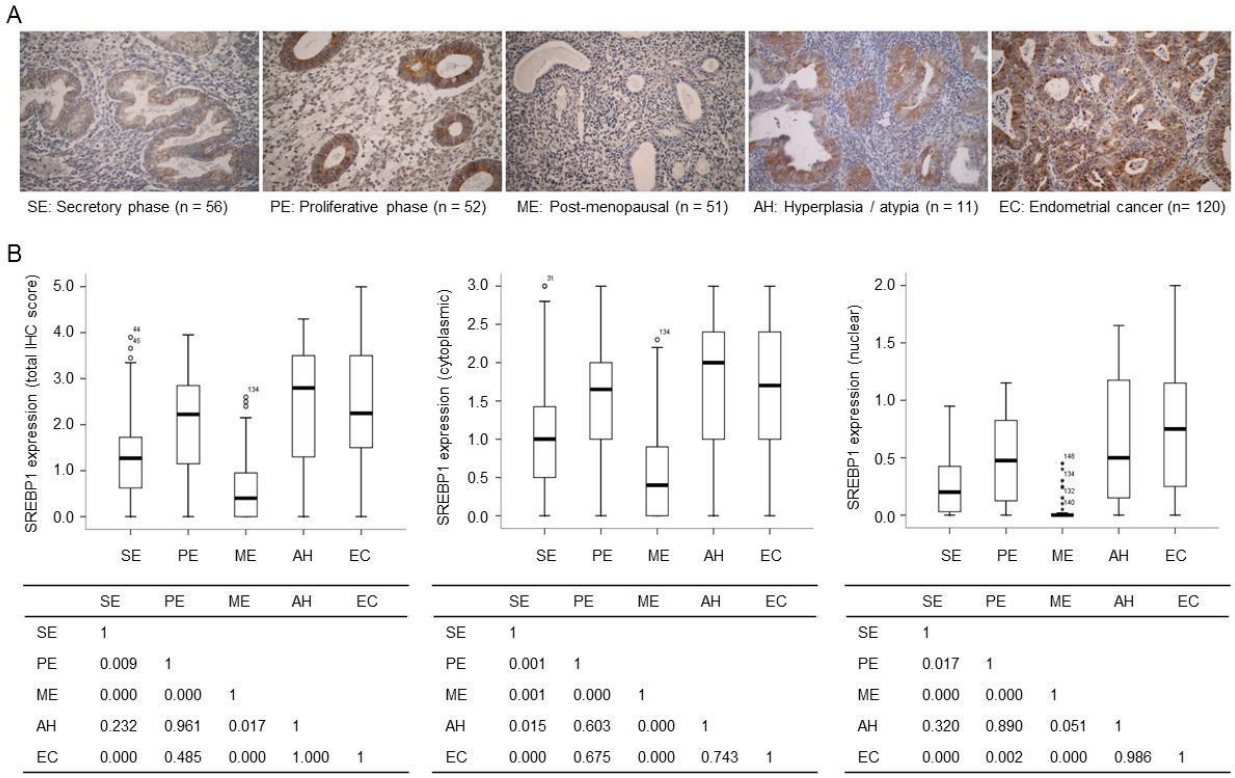


Figure 1. SREBP1 expression in endometrial cancer (EC) determined by IHC.
(A) Immunohistochemistry (IHC) staining of endometrial cancer specimens and matched adjacent non-cancerous tissues for SREBP1 protein expression and subcellular localization.
(B) Boxplot of IHC staining score for SREBP1 in whole cell, cytoplasm and nucleus in cancer and matched non-cancerous tissues.
(C) Boxplot of IHC staining score for SREBP1 in whole cell, cytoplasm and nucleus in all cancer specimens recruited to this study and non-cancerous endometrial tissues.



	SE	PE	ME	AH	EC
SE	1				
PE	0.009	1			
ME	0.000	0.000	1		
AH	0.232	0.961	0.017	1	
EC	0.000	0.485	0.000	1.000	1

	SE	PE	ME	AH	EC
SE	1				
PE	0.001	1			
ME	0.001	0.000	1		
AH	0.015	0.603	0.000	1	
EC	0.000	0.675	0.000	0.743	1

	SE	PE	ME	AH	EC
SE	1				
PE	0.017	1			
ME	0.000	0.000	1		
AH	0.320	0.890	0.051	1	
EC	0.000	0.002	0.000	0.986	1

Figure 2. Increased SREBP1 expression in atypical hyperplasia.

(A) IHC staining was conducted with anti-SREBP1 antibody on endometrial tissues derived from normal, hyperplasia without atypia and atypical hyperplasia. Secretory, proliferative and post-menopausal normal endometrial tissues were stained.

(B) Boxplot of IHC staining score for SREBP1 in whole cell, cytoplasm and nucleus in normal hyperplasic and cancerous tissues in all specimens recruited to this study as indicated. Statistical analysis of SREBP1 expression was performed showing the p-value for the difference among the experimental groups (bottom panels).

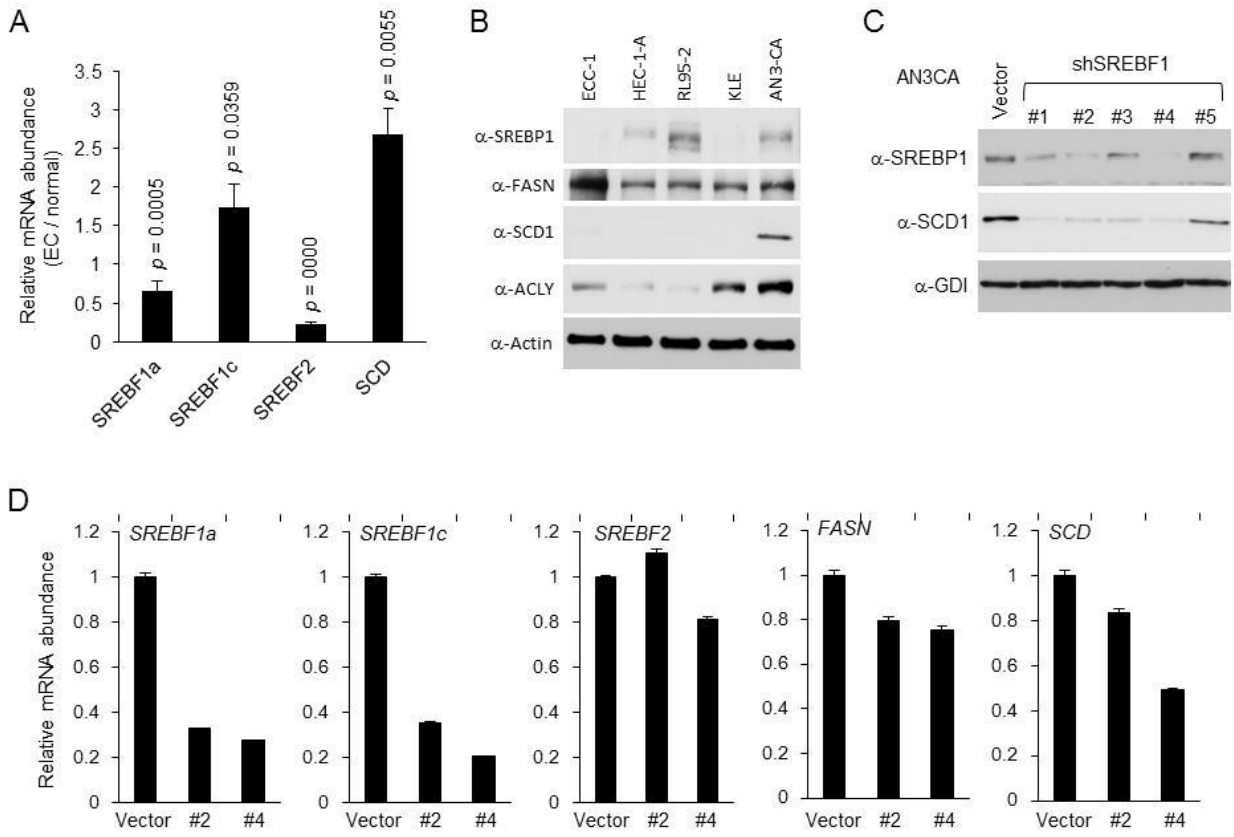


Figure 3. SREBP1 is required for lipogenic genes and cell proliferation in endometrial cancer cells.

(A) Quantitative RT-PCR analysis of mRNA abundance of SREBP1a and SREBP1c in endometrial cancer cells. RNA abundance was shown as fold change relative to that in ECC-1 cells.

(B) Western blotting (WB) analysis of lipogenic gene expression in commonly used endometrial cancer cell lines. Actin serves as protein loading control.

(C) AN3CA cells were transduced with a set of lentiviral vector expressing shRNA targeting SREBF1. Western blot analysis of SREBP1 and SCD1 were performed showing a successful knockdown of SREBP1 and reduced expression of SCD1 as a transcriptional target of SREBP1.

(D) Quantitative RT-PCR analysis of mRNA abundance of SREBP1, SREBP2 and their targets including FASN and SCD1 in #2 and #4 transduced cells.

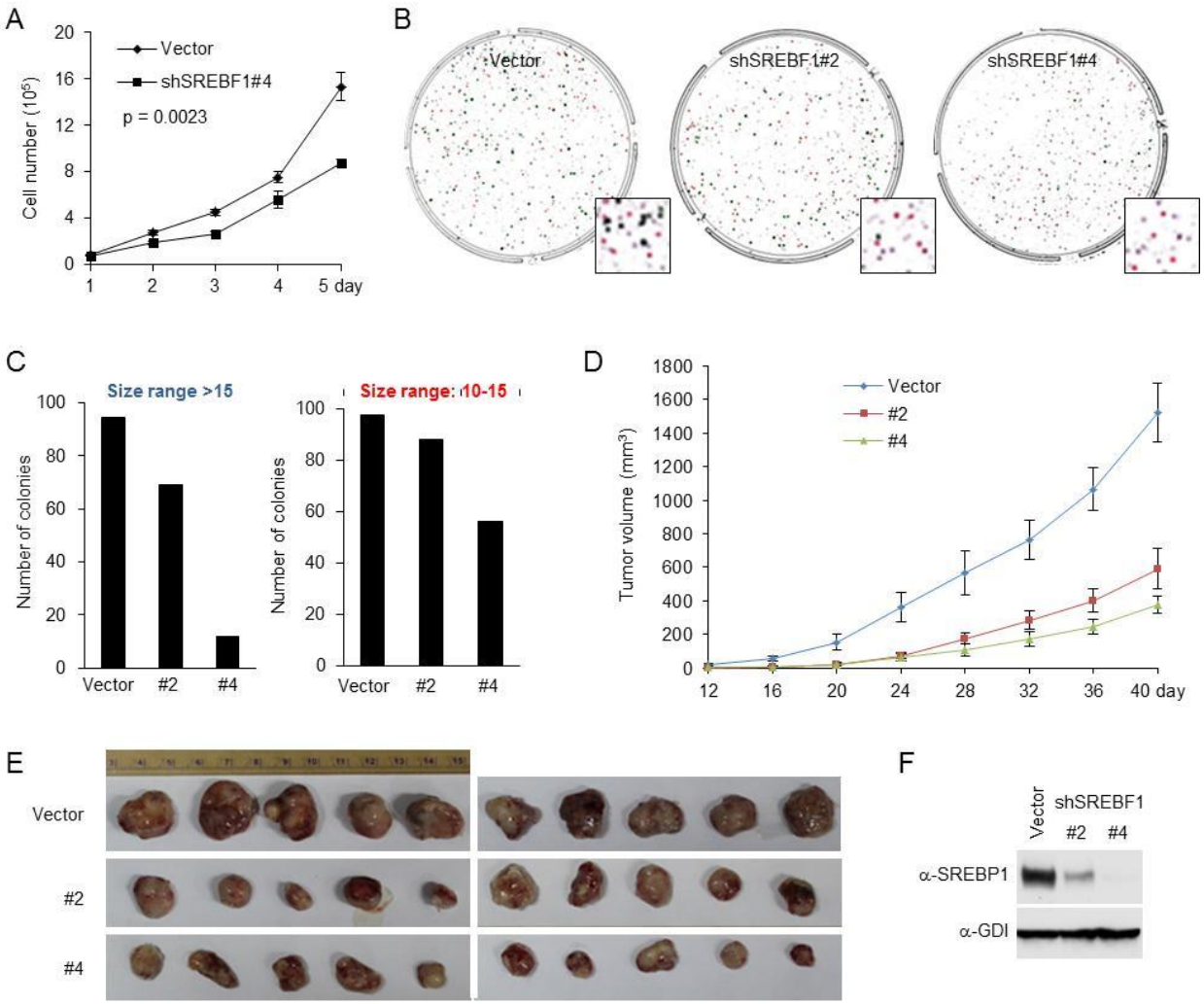


Figure 4. Knockdown of SREBP1 expression represses colony formation of endometrial cancer cells.

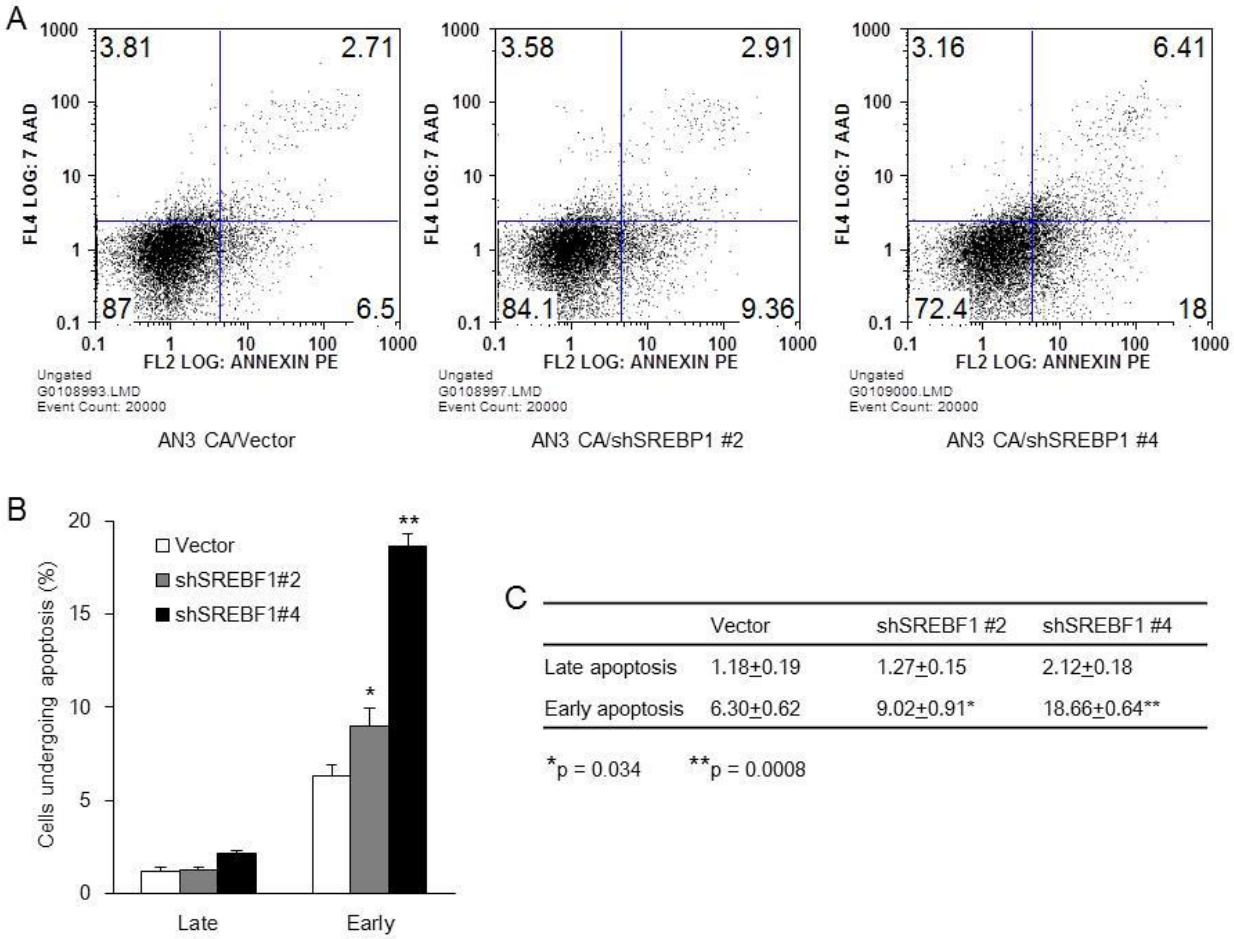
(A) 2 x 105 of AN3 CA cells were seeded per well in a 6-well cell culture device. Cellular growth was determined by counting the cells at different time points as indicated. Cells with knockdown of endogenous SREBP1 were partially defective for cell growth.

(B) Colony formation experiments were conducted in 10-cm cell culture dish. 2,000 cells were seeded and allowed to grow for 2 weeks. Medium was replaced every three days. Cells were stained with crystal violet.

(C) The number and size of colonies were calculated using software Gel-Pro Analyzer.

(D) Western blot was conducted on #2 and #4 cells, which were used for in vivo tumor growth experiment.

(E-F) AN3 CA cells (#2, #4 and vector control) were implanted into SCID mice by injecting 2 x 106 cells subcutaneously. Tumor growth was measured every four days by using a digital caliper and tumor volume was calculated.



	Vector	shSREBF1 #2	shSREBF1 #4
Late apoptosis	1.18±0.19	1.27±0.15	2.12±0.18
Early apoptosis	6.30±0.62	9.02±0.91*	18.66±0.64**

*p = 0.034 **p = 0.0008

Figure 5. Knockdown of SREBP1 promotes cell death.

(A) AN3 CA cells transduced with shSREBF1 and control vector were subjected to Annexin V analysis for apoptotic cell death.

(B) Late and early apoptotic cell death was shown as percentage to the total cells counted. Data were presented as mean ± SEM from triplicates.

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18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X_ No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X_ No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04),

the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. PACSIN 2 represses cellular migration through direct association with cyclin D1 but not its alternate splice form cyclin D1b.	Meng H, Tian L, Zhou J, Li Z, Jiao X, Li WW, Plomann M, Xu Z, Lisanti MP, Wang C*, Pestell RG*	Cell Cycle	Nov 2010	☐Submitted ☐Accepted ×Published
2. Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer.	Pan X*, Zhou T*, Tai YH*, Wang C, Zhao J, Cao Y, Zhang PJ, Yuan Chen Y, Yu M, Li HY, Li AL, Gao YF, Liang B, Zhang WN, Zhen C, Bai ZF, Gong WL, Wei LX, and Zhang XM.	Nature Medicine	Sept 2010	☐Submitted ☐Accepted ×Published
3. ChIP sequencing of cyclin D1 reveals a transcriptional role in chromosomal instability in mice.	Casimiro MC, Crosariol M, Loro E, Ertel A, Yu Z, Dampier W, Saria EA, Papanikolaou A, Stanek TJ, Li Z, Wang C, Fortina P, Addya S, Tozeren A, Knudsen ES, Arnold A, Pestell RG.	J Clin Invest.	July 2011	☐Submitted ☐Accepted ×Published
4. Repression of endometrial tumor growth by targeting SREBP1 and lipogenesis.	Li W, Tai Y, Zhou J, Gu W, Bai Z, Zhou T, Zhong Z, McCue PA, Sang N, Ji JY, Kong B, Jiang J*, and Wang C*.	Cell Cycle	May 2012	☐Submitted ☐Accepted ×Published

5. Tumor Suppressive Effects of CDK8 in Endometrial Cancer Cells.	Gu W, Wang C, Li W, Hsu F-N, Tian L, Zhou J, Yuan C, Xie X-J, Jiang T, Addya S, Tai Y, Kong B, and Ji J-Y.	Cell Cycle	July 2012	☐Submitted ☐Accepted ×Published
6. Gankyrin Activates IL-8 to Promote Hepatic Metastasis of Colorectal Cancer	Bai Z, Tai Y, Li W, Zhen C, Gu W, Jian Z, Wang Q, Lin JE, Zhao Q, Liang B, Wang C*, and Zhou T*	Cancer Res	Dec 2012	☐Submitted ☐Accepted ×Published
7. Dachshund Binds p53 To Block The Growth of Lung Adenocarcinoma Cells.	Chen K, Wu K, Cai S, Zhang W, Zhou J, Wang J, Ertel A, Li Z, Rui H, Quong A, Lisanti MP, Tozeren A, Tanes C, Addya S, Wang C, McMahon SB, Pestell RG.	Cancer Res	Aug 2012	☐Submitted ☐Accepted ×Published
8. Acetylation of the Cell-Fate Factor Dachshund Determines p53 Binding and Signaling Modules in Breast Cancer.	Chen K, Wu K, Gormley M, Ertel A, Wang J, Zhang W, Zhou J, Disante G, Li Z, Rui H, Quong AA, McMahon SB, Deng H, Lisanti MP, Wang C, Pestell RG.	Oncotarget	June 2013	☐Submitted ☐Accepted ×Published
9. Cyclin D1 Determines Estrogen Signaling in the Mammary Gland in Vivo.	Casimiro MC, Wang C, Li Z, Disante G, Willmart NE, Addya S, Chen L, Liu Y, Lisanti MP, Pestell RG.	Mol Endocrinol.	March 2013	☐Submitted ☐Accepted ×Published
10. Cyclin D1 induction of Dicer governs microRNA processing and expression in breast cancer.	Yu Z, Wang L, Wang C, Ju X, Wang M, Chen K, Loro E, Li Z, Zhang Y, Wu K, Casimiro MC, Gormley M, Ertel A, Fortina P, Chen Y, Tozeren A, Liu Z, Pestell RG.	Nat Commun	June 2013	☐Submitted ☐Accepted ×Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No ____X_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We have demonstrated that SREBP1 is significantly up-regulated and functionally activated in endometrial cancer and that SREBP1 plays important roles in endometrial cancer progression. Experimental approaches in cell culture revealed that expression of the endogenous SREBP1 is required for cancer cell growth and colony-forming capacity. Although further studies are necessary to clarify the molecular mechanisms underlying the overexpression and functional activation of SREBP1 in cancers, our results suggest that SREBP1may represent a novel therapeutic target in treating endometrial cancer.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This project also led to development of a small molecule to inhibit SREBP1 activity and tumor cell growth.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes__X__ No_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:
   Compositions and methods for treating cancer with aberrant lipogenic signaling

b. Name of Inventor(s): Chenguang Wang, Jie Zhou

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

Compositions and methods related to treatment of cancers by administering SREBP1 inhibitors.

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____X_____ No____

If yes, indicate date patent was filed: June 25, 2012

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____X_____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes__X_____ No____

If yes, how many licenses were granted?_____1_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____X_____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes ____X_____ No_____

If yes, please describe your plans:

Patent application has been filed.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
WANG, CHENGUANG, PH.D.	**Associate Professor**
eRA COMMONS USER NAME	
cxw692	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Beijing Normal University	BSc	1992	Biology
Guangzhou Medical College	MS	1995	Oncology
The Chinese Academy of Sciences	PhD	1998	Developmental Biol.
Albert Einstein College of Medicine	Postdoc	1998-2001	Oncology

Positions and Honors

2001-2002	Instructor, Albert Einstein College of Medicine
2002-2003	Instructor, Georgetown University
2003-11/2005	Assistant Professor, Georgetown University
2005-2008	Assistant Professor, Department of Cancer Biology, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA
2008-2010	Assistant Professor, Department of Stem Cell Biology & Regenerative Medicine, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA
2010-current	Associate Professor, Department of Stem Cell Biology & Regenerative Medicine, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA
2008.09-current	Adjunct Professor, Shandong Medical College, Shandong University, Jinan, China

Honors

2001-	Quest Diagnostics Young Investigator Travel Grant Award
2002	Endo-Society Travel Grant Award.

C. Publications (selected from 95 peer-reviewed publications including 43 research articles and 11 reviews/book chapters since Dr. Wang started his independent research in 2006)

1. Hulit J, Wang C, Li Z, Albanese C, Rao M, Di Vizio D, Shah S, Byers SW, Mahmood R, Augenlicht LH, Russell R, Pestell RG. Cyclin D1 Genetic Heterozygosity Regulates Colonic Epithelial Cell Differentiation and Tumor Number in ApcMin Mice. **Mol Cell Biol.** 2004 Sep; 24(17): 7598-611.
2. Wang C, Fan S, Li Z, Fu M, Rao M, Ma Y, Lisanti MP, Albanese C, Katzenellenbogen BS, Kushner PJ, Weber B, Rosen EM, and Pestell RG. Cyclin D1 antagonizes BRCA1 repression of estrogen receptor alpha activity. **Cancer Res.** 2005 Aug 1; 65(15):6557-67.
3. Li Z, Wang C, Jiao X, Lu Y, Fu M, Quong AA, Dye C, Yang J, Dai M, Ju X, Zhang X, Li A, Burbelo P, Stanley R, Pestell RG Cyclin D1 regulates cellular migration through the inhibition of thrombospondin-1 and ROCK signaling. **Mol Cell Biol.** 2006 Jun; 26(11):4240-56.

4. Wang C, Li Z, Lu Y, Du R, Katiyar S, Yang J, Fu M, Leader JE, Quong A, Novikoff PM, and Pestell RP. Cyclin D1 Repression of NRF-1 Integrates Nuclear DNA Synthesis and Mitochondrial Function. **Proc Natl Acad Sci U S A.** 2006 Aug 1; 103(31):11567-72.
5. Ju X, Katiyar S, Wang C, Liu M, Shengwen Li S, Jiao X, Zhou J, Turner J, Lisanti MP, Russell RG, Mueller S, Ojeifo J, Chen WS, Hay N, and Pestell RG. Akt1 governs breast cancer progression in vivo. **Natl Acad Sci U S A.** 2007 May 1;104(18):7438-43.
6. Li Z, Wang C, Jiao X, Katiyar S, Casimiro MC, Prendergast GC, Powell MJ, Pestell RG*. Alternate cyclin D1 mRNA splicing modulates p27KIP1 binding and cell migration. **J Biol Chem.** 2008 Mar 14;283(11):7007-15.
7. Nan F, Lü Q, Zhou J, Cheng L, Popov VM, Wei S, Kong B, Pestell RG, Lisanti MP, Jiang J, Wang C. Altered expression of DACH1 and cyclin D1 in endometrial cancer. **Cancer Biol Ther.** 2009 Aug;8(16):1534-9. Epub 2009 Aug 8.
8. Zhou J, Zhang W, Liang B, Casimiro MC, Whitaker-Menezes D, Wang M, Lisanti MP, Lanza-Jacoby S, Pestell RG, Wang C. PPARgamma Activation Induces Autophagy in Breast Cancer Cells. **Int J Biochem Cell Biol.** 2009 Nov;41(11):2334-42. Epub 2009 Jun 26.
9. Popov VM, Zhou J, Shirley LA, Quong J, Yeow WS, Wright JA, Wu K, Rui H, Vadlamudi RK, Jiang J, Kumar R, Wang C, Pestell RG. The Cell Fate Determination Factor DACH1 is expressed in Estrogen Receptor-α (ERα) positive breast cancer and represses ERα signaling. **Cancer Res**. 2009 Jul 15;69(14):5752-60.
10. Tian L, Zhou J, Casimiro MC, Liang B, Ojeifo JO, Wang M, Hyslop T, Wang C, Pestell RG. Activation of Peroxisome Proliferator-activated Receptor Promotes Tumor Growth by Enhancing Angiogenesis. **Cancer Res.** 2009 Dec 15;69(24):9236-44.
11. Zhou J, Wang C, Wang Z, Dampier W, Wu K, Casimiro MC, Chepelev I, Popov VM, Quong A, Tozeren A, Zhao K, Lisanti MP, Pestell RG*. Attenuation of Forkhead Signaling by the Retinal Determination Factor DACH1. **Proc Natl Acad Sci U S A.** 2010 Mar 29. [Epub ahead of print]
12. Pan X, Zhou T, Tai YH, Wang C, Zhao J, Cao Y, Zhang PJ, Yuan Chen Y, Yu M, Li HY, Li AL, Gao YF, Liang B, Zhang WN, Zhen C, Bai ZF, Gong WL, Wei LX, and Zhang XM. Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer. **Nature Medicine**. 2011 Jun;17(6):708-14.
13. Casimiro MC, Crosariol M, Loro E, Ertel A, Yu Z, Dampier W, Saria EA, Papanikolaou A, Stanek TJ, Li Z, Wang C, Fortina P, Addya S, Tozeren A, Knudsen ES, Arnold A, Pestell RG. ChIP sequencing of cyclin D1 reveals a transcriptional role in chromosomal instability in mice. **J Clin Invest**. 2012 Mar 1;122(3):833-43. doi: 10.1172/JCI60256. Epub 2012 Feb 6.
14. Zhao X, Feng D, Wang Q, Abdulla A, Xie XJ, Zhou J, Sun Y, Ellen S. Yang ES, Liu LP, Vaitheesvaran B, Bridges L, Kurland IJ, Strich R, Ni JQ, Wang C, Ericsson J, Pessin JE, Ji JY, and Yang F. Conserved regulation of lipid homeostasis by CDK8-mediated control of nuclear SREBP-1 protein stability. **J Clin Invest**. 2012 Jul 2;122(7):2417-27.
15. Li W, Tai Y, Zhou J, Gu W, Bai Z, Zhou T, Zhong Z, McCue PA, Sang N, Ji JY, Kong B, Jiang J, and Wang C. Repression of endometrial tumor growth by targeting SREBP1 and lipogenesis. **Cell Cycle**. 2012 Jun 15;11(12):2348-58.