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 717-231-2825.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Theodore F. Taraschi, PhD

4. **Grant Contact Person's Telephone Number:** (215) 955-3900

5. **Grant SAP Number:** 4100054872

6. **Project Number and Title of Research Project:** Project 6 - Phenotypic and Molecular Characterization of Occult AML

7. **Start and End Date of Research Project:** 1/1/2011-12/31/2014

8. **Name of Principal Investigator for the Research Project:** Stephen C. Peiper, MD

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:

   $ 351,272.14

   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
Jiang, Bing-Hua	Professor	3% Yr 3; 29% Yr 4	$79,910
Liu, Ling-Zhi	Assistant Professor	11% Yr 3; 32% Yr 4	$36,949
He, Jun	Instructor	14% Yr 3; 100% Yr 4	$66,966
Xu, Qing	Researcher-Visiting	61% Yr 4	$21,867

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
Peiper, Stephen	PI	2%

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 awarded:
Stromal miR-148a in Breast Cancer Cell Proliferation and Tumorigenesis	x☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	11/13	$100,000	$ not funded

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 will do some further studies, obtain more preliminary data, and submit a new R21 or R01 proposal.

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

Our preliminary data indicate that stromal miR-148a plays an important role in regulating cell proliferation and tumor growth. We will further determine the role and stromal miR-148a in breast tumor angiogenesis. Given the importance of microenvironment in radiation therapy, we will also study the role and mechanism of miR-148a in radiotherapy.

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				
Unknown				
Total				**1**

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

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				1
Other				
Unknown				
Total				**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 money is very helpful to get preliminary data for further funding applications.

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_____ No____x____

If yes, please describe the collaborations:

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

Yes_____ No____x_____

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

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.

Phenotypic and Molecular Characterization of Occult AML

The high incidence of relapse in acute myelogenous leukemia (AML) observed after induction of complete remission (CR) is thought to be due to the persistence in a protective niche in the bone marrow (BM). The residual AML cryptic cells are a rare population with self-renewal potential, representing Minimum Residual Leukemic Myeloblasts (MRLM). Homing of these AML cells in the BM is largely mediated through the interaction of the chemokine receptor CXCR4 expressed by AML cells with its ligand CXCL12 expressed by BM stromal cells. The objective of this project is to establish a rationale for mobilization of AML occult cells from the BM by treating patients in CR with a CXCR4 antagonist and demonstrating that AML precursor cells will be detected in bone marrow and peripheral blood as early predictor or relapse using cytogenics, multiparameter flow cytometry and next generation DNA sequencing.

Project Overview

The overall hypothesis of this proposal is that inhibition of CXCR4 could induce mobilization into peripheral blood of AML stem cells (AML-SC) hosted in the BM that constitute minimal residual disease during hematologic remission in patients with AML following stem cell transplantation (SCT). The objective of this project is to establish CXCR4 as a target for mobilization of AML occult cells in patients in CR after chemotherapy and allogeneic SCT and to establish the phenotypic and molecular signature of these AML myeloblats and AML-SC. Bone marrow and peripheral blood samples will be collected in a longitudinal study at 28, 90 and 180 days following SCT.

Expected Outcomes

In Specific Aim 1, the identification of (recipient) leukemic myeloblasts will be performed using routinely performed techniques, including morphologic analysis and detection of cytogenetic abnormalities present at the time of the initial diagnosis and/or sex chromosome conversion following SCT using fluorescence *in situ* hybridization (FISH).

In Specific Aim 2, the identification and quantification of leukemic myeloblasts will be achieved by multiparameter flow cytometric detection of the abnormal phenotype present at the time of diagnosis using a panel of antibodies specific for myeloid markers and non-myeloid differentiation markers, as well as antigens associated with AML-SC such as CD34, CD117 (c-kit), CD123 (IL-3R alpha chain), CD47, and CXCR4.

In Specific Aim 3, the quantification of leukemic myeloblasts will be based on the analysis of hematopoietic chimerism using molecular genetic analysis of short tandem repeats (STR) alleles from donor and recipient genotypes. This will be achieved with the PowerPlex 16 assay routinely

used to monitor chimerism following SCT. This assay consists of a set of multiplex PCR amplification reactions and analysis of the length of the DNA fragments will be performed on an ABI3130 Genetic Analyzer. However, this traditional method offers a limited sensitivity due to the limited threshold of detection of traditional sequencers. The sensitivity of detection and the accuracy of quantification of chimerism will be extended by using next generation sequencing (Roche 454) to analyze the products of the PowerPlex 16 assay.

These specific aims will provide the tools to address the question as to whether mobilizing AML cells into peripheral blood and thereby sampling the entire marrow organ would be a reasonable strategy to detect residual or recurrent AML at an earlier time point than conventional marrow analysis.

Summary of Research Completed

The objective of the research project is to identify residual leukemic myeloblasts and leukemia stem cells in the peripheral blood of patients with Acute Myelogenous Leukemia (AML) after heterologous Stem Cell Transplant (SCT). This requires the development of methods capable of detecting, with high sensitivity, an extremely small number of AML cells from the patient in blood samples consisting mostly of cells from the donor.

We have focused on the strategy introduced in Specific Aim 3, which uses genetic markers to discriminate the hematopoietic cells coming from the recipient from those coming from the donor. This strategy is based on the concept that emergence of recipient's cells after SCT represents an early indication of impending hematologic relapse. For these studies of mixed chimerism, we proposed to adapt the PowerPlex® 16 assay (Promega) to the Roche 454 platform of next-generation sequencing (NGS). The PowerPlex® 16 assay consists of 16 polymorphic loci containing variable numbers of tandem repeats of 4 or 5 base pairs. Pairs of primers specific for each locus are used to amplify by PCR, using genomic DNA as a template, a fragment of DNA that comprises the repeat region. The length of that fragment depends on the number of repeats and is determined by analyzing the PCR product on a DNA sequencer, such as the ABI 3130 Genetic Analyzer. Separate analysis of genomic DNA from recipient and donor origin identifies the loci that can be used to discriminate the recipient and the donor (i.e. the loci where donor and recipient have at least one different allele). Although very specific, this technology offers limited sensitivity for the detection of the minor alleles in a mixed sample (at least 2%).

Roche 454 NGS offers the possibility to analyze large numbers of individual sequences in a single run. This allows the detection and quantification of rare genetic variants and should lend itself to the analysis of mixed populations of polymorphic alleles. The possibility to use 454 NGS to analyze mixed chimerism using the PowerPlex® 16 assay was determined in five de-identified AML patients who had relapsed following SCT. Sequential archived DNA samples previously studied using the PowerPlex® 16 assay were obtained from each patient at different time points (2 to 4) after SCT. Each case included at least one sample obtained shortly after SCT and one sample obtained after clinical relapse. When available, 1 or 2 additional DNA sample(s) collected before relapse were included. Two or 3 informative loci were used for each patient.

The preparation of the samples for 454 sequencing included 4 steps:

1) Primers specific for each locus and identical to those included in the Promega PowerPlex® 16 kit were used to amplify genomic DNA using the reagents and conditions recommended by Promega. PCR products were analyzed on a Bioanalyzer 2100 (Agilent).
2) For each patient, PCR products were purified and quantified. For each time point, the PCR products corresponding to the different loci (2 per patient) were mixed in equimolar amounts and used to prepare a library for which PCR products were end-repaired and ligated to an oligonucleotide incorporating an adaptor sequence, followed by a specific sequence (MID) that functions as a barcode to identify the sample during sequencing. Each time point was prepared using a different MID to conduct multiplexed sequencing and analyze separately the data for each time point. After ligation, the products were purified from the unligated oligonucleotides, quantified, and the different time points were mixed in equimolar quantities to prepare the library.
3) The library was diluted so that statistically, each sequencing bead was conjugated to only 1 DNA fragment. After addition of DNA to the sequencing beads, DNA was clonally amplified by emulsion PCR (emPCR) in which each DNA bead is included into an individual micro-reaction chamber within the emulsion. After emPCR, the emulsion was broken and the beads carrying some DNA were magnetically separately from the empty beads.
4) The DNA beads were loaded onto a pico-titer plate according to Roche-454 protocol and the plate was installed on a 454 Junior sequencer. Reads were sorted according to their MID and analyzed using CLC Genomics Workbench (CLC bio). Mapping was performed against all the existing alleles of each locus.

The number of usable reads for each sequencing reaction ranged from 62,000 to 148,000, distributed between the 2 loci and the 2-4 time points. A total of 6 loci were used for 5 patients (D5S818, D7S820, D8S1179, D13S317, D16S539 and TPOX) and in most cases, the results were in agreement with the PowerPlex® 16 data (Figure 1). In the post-relapse samples, a decrease of the donor-specific allele(s) was observed compared with the pre-relapse samples, while a simultaneous increase of the recipient-specific allele(s) was noted. However, for each sample, a percentage of the reads had a number of repeats that did not correspond to alleles present either in the donor or the recipient. These reads are related to the “stuttering” of the polymerase that occurs in presence of repeats. Most of the stutters are 1 repeat shorter than the template (down-stuttering) and some are 1 repeat longer (up-stuttering) (see for instance patients AML-BME-007 and AML-BME-009, Fig 1). A calculation of the percentage of stuttering can be introduced, but this problem could compromise the sensitivity of the detection of rare events that could otherwise be achieved by 454 sequencing.

If the problem cannot be resolved, alternative approaches will be used. First, Roche 454 recently released a kit for high-resolution HLA genotyping. Since donor and recipient of SCT are usually an incomplete match, this system could be used to discriminate the cells of donor and recipient origin. Another approach could be the search for Single Nucleotide Polymorphisms (SNPs) that would be different between donor and recipient. Since no data is available for the patient or the donor, both DNA would have to be screened first to identify informative SNPs. When this is performed, 454 sequencing should be able to detect a small percentage of the recipient’s allele.

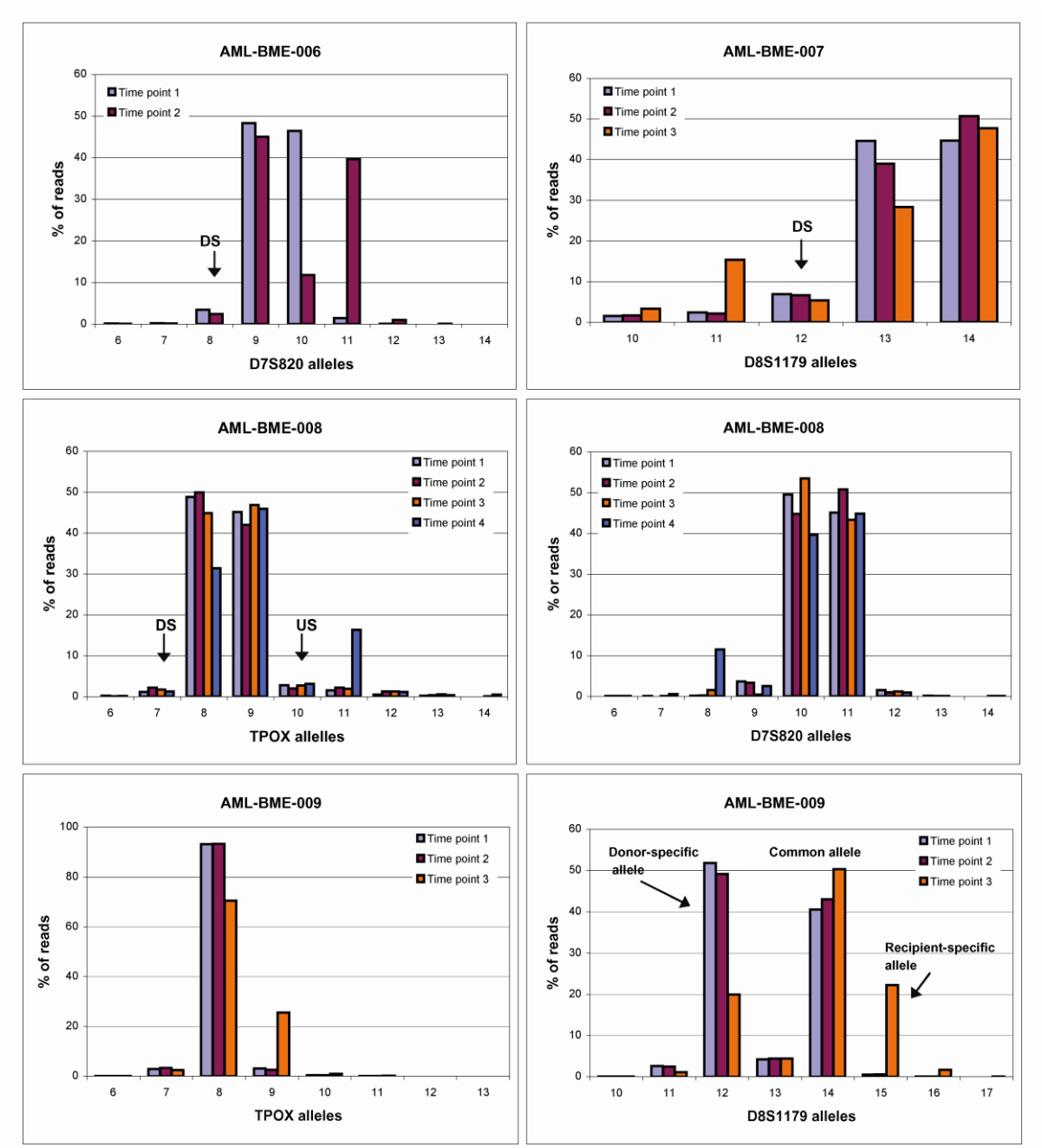

Figure 1: Adaptation of the PowerPlex 16 Assay to the Roche-454 NDG platform. Data for patients 006, 007, 008 and 009 illustrate the capacity to effectively detect and quantify alleles specific for the donor and the recipient. For each patient, the last time point is after clinical relapse. Data indicate the presence of down-stuttering (DS) and up-stuttering (US) corresponding to reads with lower or higher repeat numbers compared to the actual alleles present in the sample. Data are in accordance with PowerPlex 16 data indicating percentages of recipient cells of 86% for patient 006, 40% for patient 007, 32% for patient 008 and 50% for patient 009.

We only achieved our goal for Aim 3. This is primarily because of a transition in Dr Peiper's laboratory. Dr Jean-Marc Navenot, who worked under Dr Peiper's supervision for over 15 years, left the laboratory. This provided the opportunity to extend the scope of the project to explore the interaction of breast cancer cells with supporting stromal cells in collaboration with Dr Bing-Hua Jiang, who joined the Department of Pathology, Anatomy & Cell Biology. The new experiments explore mechanisms responsible for the effect of stromal cells on breast cancer cells, including proliferation, angiogenesis, and metastasis, again focusing on a chemokine-receptor paracrine signaling system, as outlined in the additional specific aims:

The overall hypothesis of this additional work is that inhibition of CXCR4 could induce mobilization into peripheral blood of AML stem cells (AML-SC) hosted in the BM that constitute minimal residual disease during hematologic remission in patients with AML following stem cell transplantation (SCT). The goal of these aims is to establish CXCR4 as a target for mobilization of AML occult cells in patients in CR after chemotherapy and allogeneic SCT and to establish the phenotypic and molecular signature of these AML myeloblats and AML-SC. Bone marrow and peripheral blood samples will be collected in a longitudinal study at 28, 90 and 180 days following SCT.

<u>Specific Aim 4</u>. *Determine the role and mechanism of miR-148a expression in CAFs via CCL5 expression in regulating cell proliferation.* We will use molecular biological methods to change certain molecule in breast cancer cells or CAFs. We will co-culture cancer cells and CAFs to test the effect of cancer cells and CAFs interaction. We will test cell proliferation using MTT assay.

<u>Specific Aim 5.</u> *Determine the roles of stromal miR-148a expression in regulating tumor growth and angiogenesis.* We will test stromal miR-148a in regulating breast cancer tumor growth and angiogenesis. We will use animal model to perform tumor growth assay and angiogenesis assay.

The objective of this research is to study the role and mechanism of stromal miR-148a in regulating breast cancer cell proliferation, tumor growth and angiogenesis. We achieved our goals to finish Aims 4 and 5. The methods used on Aims 4 and 5 are shown in figure legends. The results we obtained are as following:

<u>1. Co-culture of MDA-MB-231 and stromal CAF cells led to miR-148a downregulation and increased CCL5 secretion, which can suppress miR-148a expression in CAFs.</u>
Our recent results showed miR-148a acted as tumor suppressors by targeting IGF-IR and IRS1, and downregulation of miR-148a in breast cancer cells was due to the DNA methylation [1]. We found that the levels of miR-148a in CAF cells were more than 10-fold higher than those in breast cancer cells, and more than 8-fold higher than those from the co-cultured breast cancer MB-231 and CAF cells (Fig. 2A). To understand whether the co-culture of MB231 and CAF cells led to significant decrease of miR-148a expression through certain cytokine production, we performed cytokine microarray analysis, and found levels of four secreted cytokines: CXCL1, IL-6, IL-8, MCP-1 and CCL5 in the conditioned medium prepared from the co-culture cells were higher than those prepared from MB-231 or CAF cells alone. The most dramatic change was levels of CCL5 (data not shown). The mRNA levels of these cytokines in the cells using qRT-PCR analysis showed similar higher levels of CXCL1, IL-6, MCP-1, while CCL5 was 20-fold higher in the co-cultured cells than the BC and CAF cells alone (Fig. 2B). The secreted CCL5 in

the co-cultured supernatant was 7-fold higher than BC and CAF cells alone by ELISA assay (Fig. 2C). To determine whether CCL5 results in miR-148a downregulation in CAF cells, we treated CAF cells with CCL5 and found that after the treatment for 4 and 12 h, the miR-148a levels in CAF cells significantly decreased (Fig. 3).

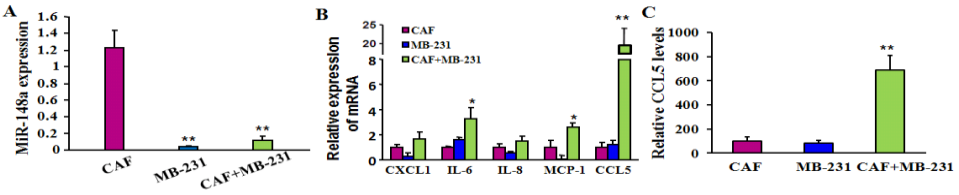

Fig. 2. Co-culture of MDA-MB-231 and stromal CAF cells led to miR-148a downregulation and increased CCL5 secretion. ***A***, CAF, MDA-MB-231 (MB-231) cells, and co-cultured cells (the ratio of CAF to MB231 was 1:2) were seeded in the 6-well plate, After 48 h, miR-148a was tested by qRT-PCR. ***B***, The cells were seeded as above in 6 well plates for 24 h, cells were cultured in serum-free medium for 48 h. Relative expression of CXCL1, IL-6, IL-8, MCP-1 and CCL5 mRNA in each group was determined by real-time PCR. The data represent mean±SD of three independent experiments. * p<0.05 and ** p<0.01, respectively. ***C***, The conditioned media were collected and subjected to ELISA assay and normalized to the cell number and CAF group. * p<0.05. (n=26).

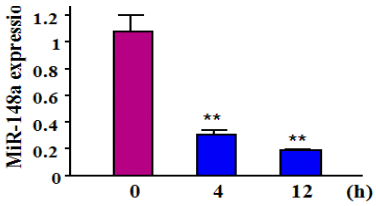

Fig. 3. CCL5 treatment decreased miR-148 expression in CAF cells. CAF cells were treated with CCL5 at 10 ng/ml for 0, 4 and 12 h. MiR-148a was determined by real-time PCR. The data represent mean±SD of three independent experiments. ** p<0.01.

2. Co-culture activated AKT and ERK1/2signaling pathways.

Our preliminary data showed that CCL5 treatment suppressed CAF miR-148a expression (Fig. 3). To study whether miR-148 downregulation in CAF is due to CCL5 induction and secretion in the co-cultured cells, we will first use neutralized antibody against CCL5 in the co-culture of MB-231 and MCF7 with CAF cells and test miR-148a expression by qRT-PCR similarly as Fig. 3. Our preliminary data (Figs. 2B and 2C) suggest that the interaction between cancer cells and CAF increases CCL5 expression. Only a little information is available for CCL5 regulation. Previous studies demonstrated that CCL5 could be transcriptionally induced by c-Jun in cancer cells and stromal cells [2,3]. We found that AKT and ERK1/2 were activated under the co-culture (Fig. 4). To examine whether AKT and ERK1/2 are responsible for the increased CCL5 secretion in the co-culture, we will test whether inhibition of AKT and ERK1/2 by their inhibitors, and knockdown of AKT and ERK1/2 using AKT and ERK1/2 siRNAs in BC cells and CAF cells to test whether these treatments inhibit CCL5 secretion.

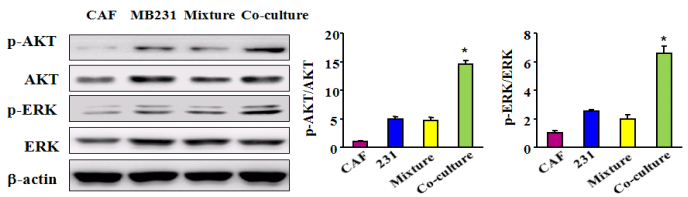

Fig. 4. Co-culture activated AKT and ERK1/2 signaling pathways. MDA-MB-231 and CAF cells were co-cultured for 48 h. Cell pellets were subjected to immunoblotting. The data represent mean±SD of three independent experiments. * p<0.05.

3. Co-culture increased cell proliferation.

To further determine the effect of interaction between breast cancer cells and CAFs, we found that co-culture led to faster cell proliferation than culturing BC or CAF cells alone (Fig. 5). These new results indicate that stromal miR-148a and CCL5 expression is important for cell proliferation.

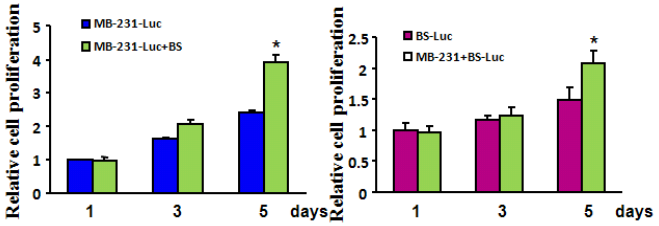

Fig. 5. Co-culture of MB-231 and CAF cells increased proliferation of MB-231 and CAF cells. MDA-MB-231 cells ($3x10^5$) stably expressing Luc construct were seeded alone or with breast stromal CAF cells ($1.5x10^5$) in 6 wells plate (left). $1.5x10^5$ breast stromal cells stably expressing Luc were seeded alone or with $3x10^5$ MDA-MB-231 cells in 6 wells plate (right). The luciferase activities were measured in day 1, 3 and 5 after cell seeded. The data represent mean±SD of three independent experiments. * p<0.01.

4. Stromal miR-148a could be secreted into exosomes for entering cancer cells, and miR-148 overexpression in caner cells inhibited cell proliferation.

To understand how stromal miR-148a exhibits its function in regulating tumorigenesis, we found that the levels of miR-148a in MB-231 cells cultured in conditioned medium prepared from CAFs expressing miR-148a (CM-CAF-miR-148a) were 76-fold compared to control MB-231 cells (Fig. 6A). The miR-148a levels in exosomes from conditioned medium of CAFs expressing miR-148a were 125-fold compared to those from SCR-control cells (Fig. 6B), suggesting that miR-148a generated by CAFs can be secreted into exosomes for entering MB-231 cells. To study the role of miR-148a in cancer cells for mediating cell proliferation, we found that forced expression of miR-148a in breast cancer cells attenuated cell proliferation (Fig. 6C). These results indicate that stromal miR-148a secretes into exosomes for entering breast cancer cells, thus regulating cancer cell proliferation.

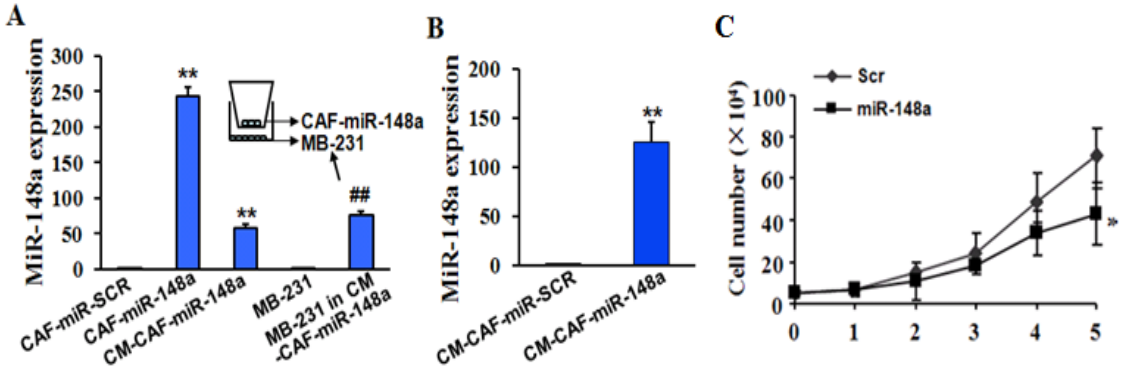

Fig. 6. Stromal miR-148a could be secreted into exosomes for entering cancer cells, and forced expression of miR-148a in MB-231 cells suppressed cell proliferation. ***A***, MiR-148a expression in stable CAFs expressing scrambled control (CAF-miR-SCR) or miR-148a (CAF-miR-148a), in conditioned medium of CAF-miR-148a (CM-CAF-miR-148a), in MB-231 cells, and MB-231 cells cultured in CM-CAF-miR-148a was determined by real-time PCR. The data represent mean±SD of three independent experiments. **, p<0.01 vs CAFs alone; ##, p<0.01 vs. MB-231 cells alone. ***B***, The supernatant was collected from CAF-miR-SCR and CAF-miR-148a stable cells. RNAs were extracted by ExoMir Kit (Bioo Scientific) and miR-148a was determined by RT-qPCR. U6 levels were used as internal control **, p<0.01.

5. CCL5 treatment suppressed miR-148a through DNA hypermethylation.

Our preliminary studies found that DNA hypermethylation is one of the mechanisms for miR-148a downregulation in BC cells [1]. To study whether CCL5 treatment regulates the enzymes involved in DNA hypermethylation, we found that CCL5 treatment at 1 and 10 ng/ml induced DNA methyltransferase 1 (DNMT1) expression (Fig. 7A), and 10 ng/ml of CCL5 increased methylation status in miR-148a promoter region in CAFs (Fig. 7B and C).

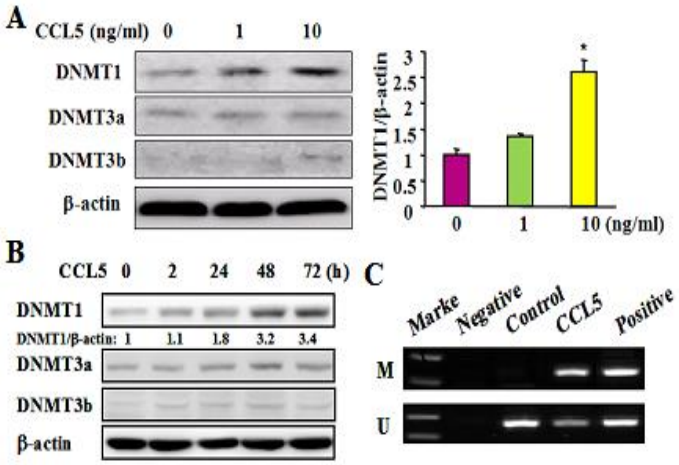

Fig. 7. CCL5 treatment increased DNMT1 expression and methylation of miR-148a promoter region in CAFs. ***A***, CAFs were treated without or with CCL5 for 12 h. The expression of DNMT1, DNMT3a, DNMT3b and β-actin was determined by immunoblotting. The results were repeated three times and the relative density was analyzed by ImmageJ software and normalized to that of β-actin. * $p<0.05$. ***B***, CAFs were treated with 10 ng/ml CCL5 for different time points and proteins were determined by immunoblotting. ***C***, CAFs were treated with or without 10 ng/ml CCL5. *Methylation-specific PCR* (MSP) analysis was performed to test the methylation status of miR-148a promoter region. U, unmethylated status; M, methylated status.

6. EGR1 induced miR-148a expression at transcriptional level.

To understand how miR-148a suppression may be regulated in cancer cells, we searched for potential transcription factors involved in miR-148a regulation within its 5-kilobase promoter regions. We found that the miR-148a promoter contains three consensus binding sites for EGR1 at positions -1503 to -1493, -1075 to -1065, and -593 to -582. EGR1 is known to bind to a GC-rich canonical sequence (GCGG[T]GGGCGG) [4,5]. To experimentally confirm that miR-148a is regulated by EGR1, we cloned four different fragments of miR-148a promoter into pGL3 luciferase reporter plasmid. With these constructs, we observed increases in luciferase activities compared to the pGL3-basic vector (Fig. 8A). To further confirm the role of EGR1 in regulating miR-148a expression, we found that overexpression of EGR1 increased miR-148a expression, whereas siRNA against EGR1 suppressed miR-148a expression using Taqman real-time PCR (Fig. 8B).

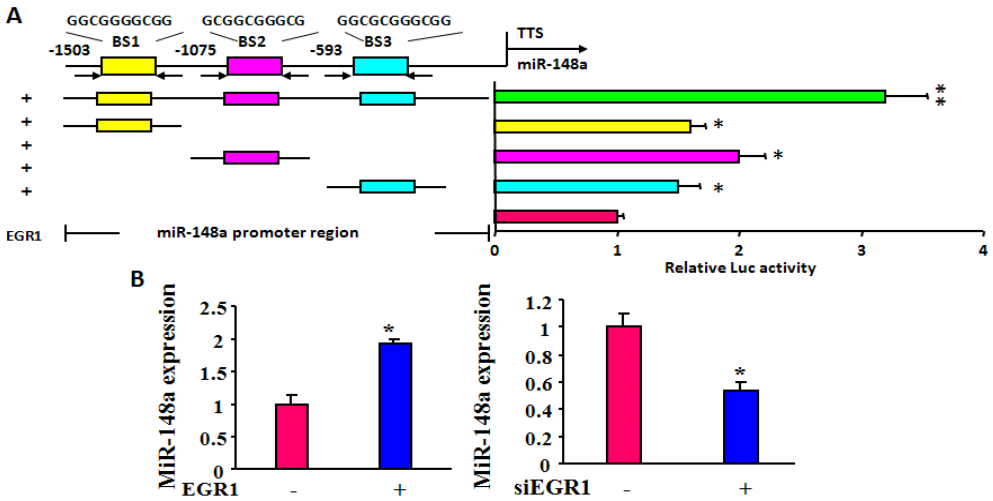

Fig. 8. EGR1 induced miR-148a expression at transcriptional level. *A,* schematic diagram shows the genomic context of miR-148a promoter. Boxes indicate the distribution of the putative EGR1 binding sites (BS). The constructs of miR-148a promoter luciferase reporters were verified by sequencing. 293T cells were co-transfected with each reporter plasmid, EGR1 expression plasmid and β-gal plasmid. The empty plasmid was used as control. The luciferase activities were analyzed (mean±SE, n=3). * p<0.05; **, p<0.01. ***B,*** 293T cells were transfected with plasmid encoding EGR1 or empty plasmid; with siRNAs against EGR1 or scrambled control (Scr). MiR-148a levels were tested by RT-qPCR (mean±SE, n=3). * p<0.01.

7. Stromal miR-148a inhibited CAFs-induced tumor growth and angiogenesis.

To study the role of miR-148a in breast cancer growth in vivo, tumor growth assay using nude mice was performed. Breast cancer cells MDA-MB-231 cells alone, cancer cells mixed with CAFs overexpressing miR-control or miR-148a by transfection were injected subcutaneously into six-week old female nude mice. Four weeks later, mice were sacrificed and the tumor tissues were trimmed out and weighed. We found that co-implantation of MB-231 and CAFs promoted tumor growth, whereas overexpression of miR-148a in CAFs suppressed tumor growth (Fig. 9A and B), and angiogenesis (Fig. 9C).

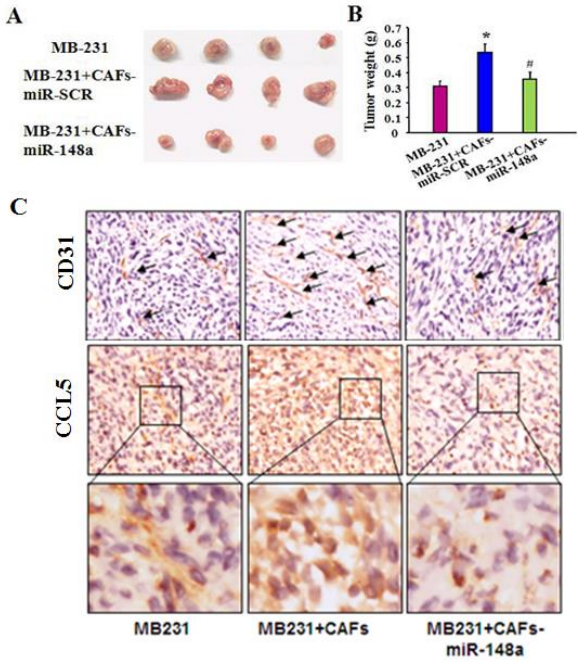

Fig. 9. Overexpression of miR-148a in CAFs inhibited CAF-induced tumor growth and angiogenesis. CAF cells were infected with lentivirus carrying scrambled sequence (SCR) or miR-148a, and selected with puromycin. MDA-MB-231 cells ($1x10^6$) alone, MDA-MB-231 cells and CAFs ($1x10^6$) were mixed and subjected to tumor growth assay using nude mice model. Mice were sacrificed and xenografts were trimmed out and weighed after 4 weeks. The representative pictures of tumors are shown in ***A***, and the weights of tumor are shown in ***B***. *, p<0.05 vs. MB-231 cells only; #, p,0.05 vs. MB-231 and CAF-miR-SCR group. ***C***. Immunohistochemistry of CD31 and CCL5 in tumor tissues.

References

[1] Xu Q, Jiang Y, Yin Y, Li Q, He J, Jing Y, Qi YT, Xu Q, Li W, Lu B, Peiper SS, Jiang BH, Liu LZ. A regulatory circuit of miR-148a/152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. *J. Mol. Cell Biol.* 2013, 5: 3-13.

[2] Mi Z, Bhattacharya SD, Kim VM, Guo H, Talbot LJ, Kuo PC. Osteopontin promotes CCL5-mesenchymal stromal cell-mediated breast cancer metastasis. *Carcinogenesis* 2011, 32:477-487.

[3] Jiao X, Katiyar S, Willmarth NE, Liu M, Ma X, Flomenberg N, Lisanti MP, Pestell RG. c-Jun induces mammary epithelial cellular invasion and breast cancer stem cell expansion. *J. Biol. Chem.* 2010, 285:8218-8226.

[4] Liu C, Rangnekar VM, Adamson E, Mercola D. Suppression of growth and transformation and inductionof apoptosis by EGR-1. *Cancer Gene Ther* 1998; 5**:** 3-28.

[5] Portales-Casamar E, Thongjuea S, Kwon AT, Arenillas D, Zhao X, Valen E *et al*. JASPAR 2010: the greatly expanded open-access database of transcription factor binding profiles. *Nucleic Acids Res* 2010; 38**:** D105-D110.

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
___x__ 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):
None				☐Submitted ☐Accepted ☐Published

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

Yes___X_____ No_____

If yes, please describe your plans:

We will organize our results and submit articles to peer-reviewed publications in the future.

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.

Breast cancer (BC) is one of the common causes of cancer-related deaths in women. The emerging evidence demonstrates that tumor microenvironment plays an important role in cancer development, in which cancer-associated fibroblasts (CAFs) are considered to be one of the key cell types to be involved in the process. However, the role and mechanism of CAF cells in human cancer development still remain to be elucidated. Recent studies have also revealed that several microRNAs (miRNAs) such as miR-125b, miR-145, miR-21, and miR-155 are deregulated in BC. Our preliminary study showed that miR-148a is downregulated in

BC tissues and cells, which may serve as a tumor suppressor by targeting IGF-IR and IRS1. Preliminary results also indicated that CAFs express much higher levels of miR-148a that are significantly diminished by the co-culture of BC and CAFs. We identified CCL5 induction as a potential factor for suppressing stromal miR-148a expression. Thus, we hypothesize that the interactions of breast cancer cells and stromal CAFs induces secreted CCL5 production, which in turn suppresses stromal CAF miR-148a expression for inducing tumor growth, metastasis, radiation response, and angiogenesis. The results obtained from the project will establish a new conceptual base for better understanding breast cancer development mediated by stromal CAFs, and create a new paradigm that stromal CCL5 induction and miR-148a downregulation are important in regulating breast cancer growth, metastasis and angiogenesis. We will also establish a chimeric tumor model for studying novel interactions of cancer cells and CAFs via stromal CCL5 receptor CCR5 in inducing tumor angiogenesis. We believe that the accomplishment of this project would provide a novel concept and information in understanding breast cancer development.

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.

We found that stromal miR-148a/CCL5 pathway plays an important role in breast cancer development.

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_____ No___x_____

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:

b. Name of Inventor(s):

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

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_____ No___

If yes, indicate date patent was filed:

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___
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_____ No___

If yes, how many licenses were granted?_____

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

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_____ No___x_____

If yes, please describe your plans:

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; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME Stephen C. Peiper, MD	POSITION TITLE Chairman and Peter A. Herbut Professor – Jefferson Medical College of Thomas Jefferson University
eRA COMMONS USER NAME STEPHENPEIPER	

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
Washington University, St. Louis, MO	A.B.	1969 -1973	Biology
St. Louis University, St. Louis, MO	M.D.	1973-1977	Medicine
University of North Carolina at Chapel Hill	Resident	1977-1981	Anat. & Clin. Path

A. Positions and Honors

Professional Appointments

1979-80 Fellow, Hematopathology, University of North Carolina at Chapel Hill

1980-81 Chief Resident & Clinical Instructor, Pathology, University of North Carolina at Chapel Hill

1981-83 Research Associate, Pathology, St. Jude Children's Research Hospital, Memphis, TN

1983-88 Assistant Member, Pathology, St. Jude Children's Research Hospital, Memphis, TN

1983-88 Assistant Member, Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN

1988-91 Associate Prof., Pathology, Medicine, Scientist, Cancer Center, University of Alabama, Birmingham

1991-02 Agnes Brown Duggan Professor, Depts. of Pathology, Biochemistry & Molecular Biology, Surgery, Chemistry, University of Louisville, Louisville, Kentucky

2002-08 Chairman & Edgar R Pund Professor, Department of Pathology, Medical College Of Georgia, Augusta, Georgia

2008 Chairman & Peter A Herbut Professor, Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania

B. Selected 15 peer-review publications (from more than 150 papers)

1. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, **Peiper SC,** Schall TJ, Littman DR, Landau NR. Identification of a major co-receptor for primary isolates of HIV-1. ***Nature***. 1996; 381(6584):661-66.
2. Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, **Peiper SC**, Parmentier M, Collman RG, Doms RW. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. ***Cell.*** 1996; 85(7):1149-58.
3. Dawson TC, Lentsch AB, Wang Z, Cowhig JE, Rot A, Maeda N, **Peiper SC***. Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). ***Blood*** 2000; 96(5):1681- 84.
4. Navenot JM, Wang Z, Chopin M, Fujii N, **Peiper SC***. Kisspeptin-10-induced signaling of GPR54 negatively regulates chemotactic responses medicated by CXCR4:a potential mechanism for the metastasis suppressor activity of kisspeptins. ***Cancer Res***. 2005; 65(22):10450-6.
5. Zhang W, Navenot JM, Frilot NM, Fujii N, **Peiper SC***. Association of nucleophosmin negatively regulates CXCR4-mediated G protein activation and chemotaxis. ***Mol Pharmacol*** 2007;72(5):1310-21.
6. Nash KT, Phadke PA, Navenot JM, Hurst DR, Accavitti-Loper MA, Sztul E, Vaidya KS, Frost AR, Kappes JC, **Peiper SC**, Welch DR. Requirement of KISS1 secretion for multiple organ metastasis suppression and maintenance of tumor dormancy. ***J Natl Cancer Inst***. 2007; 99(4):309-21.
7. Lipan O, Navenot JM, Wang Z, Huang L, **Peiper SC***. Heat shock response in CHO mammalian cells is controlled by a nonlinear stochastic process. ***PLoS Comput Biol.*** 2007; 3(10):1859-70.
8. Fiskus W, Rao R, Fernandez P, Herger B, Yang Y, Chen J, Kolhe R, Mandawat A, Wang Y, Joshi R, Eaton K, Lee P, Atadja P, **Peiper SC**, Bhalla K. Molecular and biologic characterization and drug sensitivity of pan-histone deacetylase inhibitor-resistant acute myeloid leukemia cells. ***Blood*** 2008;112(7):2896-905.
9. Navenot JM, Fujii N., **Peiper SC**, KiSS1 metastasis suppressor gene product induces suppression of tyrosine kinase receptor signaling to Akt, tumor necrosis factor family ligand expression, and apoptosis. ***Mol Pharmacol*** 2009;75(5): 1074-83.
10. Navenot JM, Fujii N., **Peiper SC***. Activation of Rho and Rho-associated kinase by GPR54 and KiSS1 metastasis suppressor gene product induces changes of cell morphology and contributes to apoptosis. ***Mol Pharmacol*** 2009;75(6): 1300-6.
11. Evans BJ, Wang Z, Broach JR, Oishi S, Fujii N, **Peiper SC***. Expression of CXCR4, a G-protein-coupled receptor for CXCL12 in yeast identification of new-generation inverse agonists. ***Methods Enzymol***. 2009;460:399-412.
12. Liu LZ, Jiang Y, Carpenter RL, Jing Y, **Peiper SC**, Jiang BH. Role and mechanism of arsenic in regulating angiogenesis. ***PLoS One*** 2011; 6(6): e20858.
13. Navenot JM, Evans B, Oishi S, Setsuda S, Fujii N, **Peiper SC*.** The metastasis suppressor KISS1 lacks antimetastatic activity in the C8161.9 xenograft model of melanoma. ***Melanoma Res.*** 2012; 22(2):140-50.
14. Xu Q, Jiang Y, Yin Y, Li Q, He J, Jing Y, Qi YT, Xu Q, Li W, Lu B, **Peiper SC**, Jiang BH, Liu LZ. A regulatory circuit of miR-148a/152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. ***J Mol Cell Biol***. 2013; 5(1):3-13.

BIOGRAPHICAL SKETCH

NAME Bing-Hua Jiang, PH.D.	POSITION TITLE Professor
eRA COMMONS USER NAME bhjiang	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
South China University of Tropical Crops, Hainan, China	B.S.	07/84	Plant Genetics
Mississippi State University, Starkville, MS	Ph.D.	08/94	Molecular Biology
The Johns Hopkins University School of Medicine, Baltimore,MD	Postdoctoral Fellow	03/97	Molecular Biology
The Scripps Research Institute, La Jolla, CA	Research Associate	09/00	Molecular Biology

A. Positions and Honors.

2000-2006 Assistant Professor, Dept. of Immunology, and Cell Biology and Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV

2006-2010 Associate Professor, Department Immunology, and Cell Biology and Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV

2010-present Professor, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelpha. PA

B. PUBLICATIONS (selected from 135 papers that have citations of 20,588 times; h-index: 57; i10-index: 112).

1. **Jiang, B.H.**, J.Z. Zheng, M. Aoki, and P.K. Vogt. 2000. Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of VEGF in endothelial cells. ***Proc Natl Acad Sci U S A*** **97:** 1749-1753.
2. Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi XL, **Jiang BH***. Vanadate-induced expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. ***J Biol.Chem***. 2002;277(35):31963-71.
3. Skinner HD, Zheng JZ, Fang J, Agani FH, **Jiang BH***. Vascular endothelial growth factor transcriptional activation is mediated by hypoxia-inducible factor 1alpha, HDM2, and p70S6K1 in response to phosphatidylinositol 3-kinase/AKT signaling. ***J Biol.Chem***. 2004;279(44):45643-51.
4. Peng Y, **Jiang BH**, Yang PH, Cao Z, Shi X, Lin MC, He ML, Kung HF. Phosphatidylinositol 3-kinase signaling is involved in neurogenesis during Xenopus embryonic development. ***J Biol Chem***. 2004; 279(27): 28509-28514. (Co-first authors)
5. Fang J, Xia C, Cao Z, Zheng JZ, Reed E, and **Jiang BH ***. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. ***FASEB J***. 2005;19(3):342-353.

6. Liu LZ, Hu XW, Xia C, He J, Zhou Q, Shi XL, Fang J, **Jiang BH***. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells. ***Free Radical Biology & Medicine***. 2006;41(10):1521-33.
7. Meng Q, Xia C, Fang J, Rojanasakul Y, **Jiang BH***. Role of PI3K and AKT specific isoforms in ovarian cancer cell migration, invasion and proliferation through the p70S6K1 pathway. ***Cell Signal***. 2006;18(12):2262-71.
8. Fang J, Meng Q, Vogt PK, Zhang R, and **Jiang BH *.** A downstream kinase of the mammalian target of rapamycin, p70S6K1, regulates human double minute 2 protein phosphorylation and stability. ***J Cell Physiol***. 2006; 209(2): 261-5.
9. Xia C, Meng Q, Cao Z, Shi XL, **Jiang BH***. Regulation of angiogenesis and tumor growth by p110 alpha and AKT1 via VEGF expression. ***J Cell Physiol***. 2006;209(1):56-66.
10. Fang J, Ding M, Yang L, Liu LZ, **Jiang BH***. PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis. ***Cell Signal***. 2007;19(12):2487-97.
11. Xia C, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, **Jiang BH***. Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. ***Cancer Res.*** 007;67(22):10823-30.
12. Yang PH, Cheung WK, Peng Y, He ML, Wu GQ, Xie D, **Jiang BH**, Huang QH, Chen Z, Lin MC, Kung HF. Makorin-2 is a neurogenesis inhibitor downstream of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signal. ***J Biol Chem***. 2008; 283(13): 8486-8495.
12. He J, Xu Q, Jing Y, Agani F, Qian X, Carpenter R, Li Q, Wang XR, Peiper SS, Lu Z, Liu LZ, **Jiang BH***. Reactive oxygen species regulates ERBB2 and ERBB3 expression via miR-199a/125b and DNA methylation. ***EMBO Rep.*** 2012; 13(12):1116-22.
13. Li Q, Fu GB, Zheng JT, He J, Niu XB, Chen QD, Yin Y, Qian X, Xu Q, Wang M, Sun AF, Shu Y, Rui H, Liu LZ, **Jiang BH***. NADPH oxidase subunit p22(phox)-mediated reactive oxygen species contribute to angiogenesis and tumor growth through AKT and ERK1/2 signaling pathways in prostate cancer. ***Biochim Biophys Acta***. 2013;1833(12):3375-85.
14.Wang L, Shi ZM, Jiang CF, Liu X, Chen QD, Qian X, Li DM, Ge X, Wang XF, Liu LZ, You YP, Liu N, **Jiang BH***. MiR-143 acts as a tumor suppressor by targeting N-RAS and enhances temozolomide-induced apoptosis in glioma. ***Oncotarget***. 2014; 5(14):5416-27.

C. Research Support.

Ongoing Research Support

R01-ES020868-01 12/15/13-11/30/18
NIEHS, NIH; Role: PI
Title: Reactive oxygen species-induced CXCL8 in ovarian cancer
The major goals are to study the molecular mechanism of reactive oxygen species in inducing CXCL8, ovarian tumor growth and angiogenesis

R21-CA175975 Peiper and Jiang (multi-PI) 9/1/14-8/30/16
NIH/NCI; Role: multi-PI
Reactive oxygen species-induced CXCL8 in ovarian cancer
The major goals are to determine how ROS regulate CXCL8 expression to mediate cancer development, tumor growth, and angiogenesis, in which CXCL8 plays a significant role as a paracrine factor for stimulating angiogenesis, and, potentially as an autocrine growth factor.

BIOGRAPHICAL SKETCH

NAME Ling-Zhi Liu, MD, Ph.D.	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME lxl027	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shenyang Medical College	MD	09/94	Clinical Medicine
China Medical University	MD, Ph.D.	09/00	Internal Medicine
The Third Military Medical University, Chongqing, China	Residency	12/03	Internal Medicine
Institute for Nutritional Sciences, Chinese Academy of Sciences, Shanghai, China	Postdoctoral Fellow	12/05	Molecular Biology
West Virginia University, Morgantown, WV	Research Associate	04/10	Molecular Biology

B. Positions and Honors.

Position:

04/10-04/14 Research Assistant Professor, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107

05/14-present Assistant Professor, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107

C. Selected 15 peer-reviewed publications (selected from 47 articles).

1. **Liu LZ**, Fang J, Zhou Q, Hu X, Shi X, Jiang BH. Apigenin inhibits VEGF expression and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. ***Mol Pharmacol***. 2005; 68(3): 635-43.
2. **Liu LZ**, Hu XW, Xia C, He J, Zhou Q, Shi X, Fang J, Jiang BH. Reactive oxygen species regulate EGF-induced VEGF and HIF-1α expression through activation of AKT and p70S6K1 in human ovarian cancer cells. ***Free Radic Biol Med***. 2006; 41(10): 1521-33.
3. Xia C*, Meng Q *, **Liu LZ ***, Yongyut R, Wang XR, Jiang BH. Reactive oxygen species in cancer cells regulate angiogenesis and tumor growth through expression of vascular endothelial growth factor. ***Cancer Res***. 2007; 67(22):10823-30. **(*Co-first author)**
4. Cao Z, **Liu LZ**, Dixon DA, Zheng JZ, Chandran B, Jiang BH. Insulin-like growth factor-I induces cyclooxygenase-2 expression via PI3K, MAPK and PKC signaling pathways in human ovarian cancer cells. ***Cell Signal***. 2007;19(7):1542-53.
5. Zhou Q*, **Liu LZ ***, Fu B, Hu X, Shi X, Jiang BH. Reactive oxygen species regulate insulin-induced VEGF and HIF-1α expression through the activation of p70S6K1 in human prostate cancer cells. ***Carcinogenesis***. 2007; 28 (1):28-37. **(*Co-first author)**

6. **Liu LZ**, Zhou XD, Qian G, Shi X, Fang J, Jiang BH. AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. ***Cancer Res***. 2007; 67 (13): 6325-32.
7. **Liu LZ**, Zheng JZ, Wang XR, Jiang BH. Endothelial p70S6K1 in regulating tumor angiogenesis.
Cancer Res. 2008; 68(19):8183-8138.
8. **Liu LZ**, Li C, Chen Q, Jing Y, Carpenter R, Jiang Y, Kung HF, Lai L, Jiang BH. MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1α expression. ***PLoS One***. 2011; 6(4):e19139.
9. **Liu LZ**, Jiang Y, Carpenter RL, Jing Y, Peiper SC, Jiang BH. Role and mechanism of arsenic in regulating angiogenesis. ***PLoS One***. 2011; 6(6):e20858.
10. Lin CC, **Liu LZ**, Addison JB, Ivanov AV, Ruppert JM. KLF4-microRNA-206 autoregulatory feedback loop can promote or inhibit protein translation depending upon cell context. ***Mol Cell Biol.*** 2011; 31(12):2513-27.
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12. Xu Q, Jiang Y, Yin Y, Li Q, He J, Jing Y, Qi YT, Xu Q, Li W, Lu B, Peiper SS, Jiang BH, **Liu LZ***. A regulatory circuit of miR-148a/152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. ***J Mol Cell Biol.*** 2013; 5(1):3-13. **(*Corresponding author)**
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D. Research Support.
Ongoing Research Support

R01-ES020868-01 12/15/13 -10/31/18
NIEHS/NIH; PI (Jiang)
Role: Co-I
Title: Molecular mechanism of arsenic carcinogenesis
The major goals are to study the molecular mechanism of arsenic in inducing cell transformation, tumor growth and angiogenesis.

R21-CA175975 9/1/14-8/30/16
NIH/NCI; Multi-PI (Peiper and Jiang)
Role: Co-I
Reactive oxygen species-induced CXCL8 in ovarian cancer
The major goals are to determine how ROS regulate CXCL8 expression to mediate cancer development, tumor growth, and angiogenesis, in which CXCL8 plays a significant role as a paracrine factor for stimulating angiogenesis, and, potentially as an autocrine growth factor.