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):** 1/1/2010 - 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:** 1 - Genetic Targets of Breast Tumor-Initiating Cells

7. **Start and End Date of Research Project:** 1/1/2010 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Richard G. Pestell, MD, PhD

9. **Research Project Expenses**.

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

   $ 208,524.04

   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
Wu, K	Assoc. Professor	55% Yr 2	57,932
Velasco Velazquez, M	Instructor	25% Yr 2 & Yr 3	18,644
Jiao, X	Instructor	40% Yr 4	8,692

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	Position Title	% of Effort on Project
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:
DACH1/Eya Cell-fate Determination Factor and Mammary Tumorigenesis R01 CA 132115-02	☒NIH ☐ Other federal (specify:__) ☐ Nonfederal source (specify:_)	07/01/14-06/30/19	$1,937,500	
The role of retinal determination gene network (RDGN) in hormone signaling transduction and prostate tumorigenesis	☒ NIH ☐ Other federal (specify:_____) ☐Nonfederal source (specify:_)	Under review	$697,500	$

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:

I plan to write for support from the NIH and foundations for ongoing support.

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

The interaction with YB1 is dependent upon phosphorylation of DACH. We will determine the mechanisms governing phosphorylation in order to target this process to block breast cancer stem cell expansion. We will determine whether this process is general for other types of cancers.

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	2			
Female				
Unknown				
Total	**2**			

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

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

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.

The ability to provide salaries and supplies for this project has provided the important support for our research infrastructure, which is dependent upon billing for research.

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:

This research was enabled by collaboration with:

- Department of Molecular Oncology, British Columbia Cancer Research Center, Vancouver, BC, V5Z1L3, Canada
- University of Manchester, Manchester Breast Centre, Wilmslow Road, M20, 4BX, Manchester, England, UK.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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.

Genetic Targets of Breast Tumor-Initiating Cells - Breast cancer is the second most prevalent cancer-related death in women in the United States. A subpopulation of breast tumor cells have been identified and are referred to as "breast tumor-initiating cells" or cancer stem cells (CSCs). These self-renewing CSCs were first described in the hematopoietic system, and have now been putatively identified in breast, colon and brain tumors. It has been proposed that all tumor metastasis are caused by CSCs, and it is suspected that these cells contribute to therapy resistance and tumor recurrence. The focus of this project is to determine new genetic mechanisms governing breast CSC expansion and invasion, and to identify specific secreted factors regulated by gene deletion, to provide CSC-based therapies.

Project Overview

The goal of this project is to determine new genetic mechanisms governing CSC expansion and invasion, using transgenic mice. Over the last 15 years, this laboratory has generated mammary gland-targeted gene excision/inactivation mice, and crossed these mice to mammary gland-targeted oncogene mice. These mice and the genetic material and tissues previously derived from these animals, will be used to determine the role of six specific genes, in the expansion of CSC.

Specific Aim: Determine the role of genetic determinants in CSC expansion.
The relative proportion of cells with characteristics of breast CSCs will be assessed in transgenic mice and tumor-derived cell lines by genetic deletion of Notch, Akt1, c-jun, NFκB, $p21^{Cip1}$ and DACH1, using multiple assays (self renewal, self surface markers, Aldh1 production, lineage repopulation).

Milestones

Milestone 1: Determine the role of genetic determinants in CSC expansion.

Milestone 2: Determine the role of genetic-mediated breast CSC expansion in mammary tumor onset, progression and metastasis.

Milestone 3: Determine the role of secreted factors in stem cell expansion and therapy response.

Milestone 4: Functional map of these genes and provide the identity of secreted factors in governing breast CSC expansion.

Research Progress

Milestone 1: Determine the role of genetic determinants in CSC expansion.
In these studies we demonstrated that NFKB plays an essential role in the expansion. The inhibition of NFKb by a transgene in the mammary gland in the mouse demonstrated for the first time the requirement for the NFKB pathway in the onset and progression of tumorigenesis.[10] We showed endogenous DACH1 constitutively repressed breast cancer stem cells.[11] We showed EYA, which bound DACH1 promoted BCSC expansion.[5]

We began an analysis of small non-coding RNA in cancer stem cells. We showed that EYA regulated stem cells via cyclin D1.[5] We therefore examined the non-coding (miRNA) regulated by cyclin D1 to determine candidates governing CSC expansion. We identified a cluster of miRNA regulated by cyclin D1 and showed they contributed to breast cancer cell growth and migration and that cyclin D also regulated miRNA biogenesis.[14] We are now assessing the role of this pathway in determining CSC expansion.

We identified YB-1 as a DACH1 binding protein that governs breast tumor growth and invasion.[1] The epithelial-mesenchymal transition (EMT) enhances cellular invasiveness and confers tumor cells with cancer stem cell-like characteristics, through transcriptional and translational mechanisms. The mechanisms maintaining transcriptional and translational repression of EMT and cellular invasion are poorly understood. We showed Dachshund (DACH1), suppressed EMT via repression of cytoplasmic translational induction of Snail by inactivating the Y box-binding protein (YB-1). In the nucleus, DACH1 antagonized YB-1-mediated oncogenic transcriptional modules governing cell invasion. DACH1 blocked YB-1-induced mammary tumor growth and EMT in mice. In basal-like breast cancer, the reduced expression of DACH1 and increased YB-1 correlated with poor metastasis-free survival. The loss of DACH1 suppression of both cytoplasmic translational and nuclear transcriptional events governing EMT and tumor invasion may contribute to poor prognosis in basal-like forms of breast cancer, a relatively aggressive disease subtype.[1]

As we had found that YB1 induced cyclin D1 and cyclin D1 appeared to serve at a pivotal point in the CSC EMT intersection, we examined the role of cyclin D1 in the stem cell EMT phenotype in vivo. During our analysis of the mammary gland of the *cyclin D1*$^{-/-}$ mice, studies were published indicating a key role for cyclin D1 in promoting breast stem cell expansion. We therefore extended these studies to determine whether this was a general property of cyclin D1 by examining the prostate.[4] In these prostate epithelial cells, cyclin D1 plays a clear and dramatic function to sustain stem cells and to inhibit EMT.

We examined the mechanism by which DACH1 suppresses CSC. DACH1 was reduced in breast cancer cell lines with high expression of TIC markers and in patient samples of the basal breast cancer phenotype. Re-expression of DACH1 reduced new tumor formation in serial

transplantations in vivo, reduced mammosphere formation, and reduced the proportion of CD44(high)/CD24(low) breast tumor cells.[11] Conversely, lentiviral shRNA to DACH1 increased the breast (B)TIC population. Genome-wide expression studies of mammary tumors demonstrated DACH1 repressed a molecular signature associated with stem cells (SOX2, Nanog, and KLF4) and genome-wide ChIP-seq analysis identified DACH1 binding to the promoter of the Nanog, KLF4, and Lin28 genes. KLF4/c-Myc and Oct4/Sox2 antagonized DACH1 repression of BTIC. Mechanistic studies demonstrated DACH1 directly repressed the Nanog and Sox2 promoters via a conserved domain. Endogenous DACH1 regulates BTIC in vitro and in vivo.[11]

YB-1 induction of breast cancer invasiveness and mammosphere expansion is antagonized by DACH1. Dedifferentiation through activation of the embryonic epithelial mesenchymal transition (EMT) program strongly enhances cancer cell motility and dissemination. EMT, a characteristic of embryonic development, wound healing and tissue remodeling, involves a molecular program inducing abundance of transducer proteins (SNAIL, TWIST, ZEB2), which coordinate the reduction in epithelial (E-cadherin) and induction of mesenchymal (N-cadherin, Fibronectin, Vimentin) marker proteins. A variety of extracellular signals induce EMT including transforming growth factor beta (TGFβ), and extracellular matrix components such as collagen and hyaluronic acid. Together these pathways converge on EMT-inducing transcription factors, including SNAIL, Slug, ZEB1/2, Twist, FoxC2, ID1 and Goosecoid. EMT induction of tumor cells introduces cancer stem cell (CSC)- like properties with consequent therapeutic resistance and tumor recurrence. Induction of the EMT program involves both transcriptional and translational activities, although the mechanisms coordinating these activities are not well understood.

Ectopic expression of YB-1 confers invasiveness of MCF10A cells.[10] Transmigration and cellular invasion may involve distinguishable or common processes. In order to determine whether endogenous YB-1 promotes cellular migration in MDA-MB-231 cells, shRNA to YB-1 was used to transduce cells. In order to determine whether DACH1 could antagonize YB-1 induction of invasion, 3D-matrigel invasion assays were conducted. The expression of DACH1 was sufficient to inhibit MDA-MB-231 cell transwell migration (**Fig. 1A**), matrigel invasion (**Fig. 1B**) and collagen invasion (**Fig. 1C, D**). shRNA to YB-1 similarly reduced migration and invasion (**Fig. 1A-C**).

The induction of EMT may enrich gene expression associated with ES and/or TIC. In order to determine whether YB-1 abundance may induce TIC, we deployed a widely accepted model of mammosphere formation in which the number of mammospheres reflects symmetrical division in multi-potential cells. MDA-MB-231 cells were stably transduced with Ponasterone-inducible DACH1 and/or shRNA to YB-1. Increased DACH1 expression or reduced YB-1 abundance via shRNA, reduced the number of mammospheres by >60% in multiple generations (**Fig. 1D**). shRNA to YB-1 reduced mammosphere size by ~15% (**Fig. 1E**). DACH1 expression did not further reduce the mammosphere number in the presence of YB-1 shRNA (**Fig. 1E**).

YB-1 and DACH1 expression is inversely correlated in Luminal A and Basal human breast cancer. We examined further the relationship between YB-1 and DACH1 expression in breast cancer cell line and tissues. We examined the relative mRNA levels for DACH1 in luminal

(n=15) and basal (n=14) breast cancer cell lines. DACH1 relative mRNA levels were reduced and YB-1 levels increased in basal breast cancer cell lines compared with luminal breast cancer cell lines **(Fig. 2A)**. Previous studies had demonstrated reduced DACH1 abundance in human breast cancer correlating with poor prognosis. RNA expression of DACH1 was higher in luminal cancer with decreased abundance in human basal cancer. In contrast, YB-1 levels were increased in human basal breast cancer compared with luminal cancer **(Fig. 2A)**. Immunohistochemical staining was conducted on normal human breast tissue, with comparison made to human breast cancer **(Fig. 2B)**. DACH1 was expressed in normal human tissue with reduced expression in basal breast cancer. YB-1 expression was reduced in normal breast tissue but increased in basal breast cancer **(Fig. 2C)**.

In order to determine the relative abundance of DACH1 and YB-1 in human breast cancer we examined gene expression of 2,550 breast cancer samples **(Fig. 2D)**. The tumors were sub-classified by the genetic sub-types of luminal A, luminal B, basal, normal-like and HER2 **(Fig. 1D)**. Molecular subtyping allowed us to identify an enhancement of DACH1 expression with low YB-1 expressing tumors as luminal A **(Fig. 2E)** and conversely increased YB-1 expression with low DACH1 levels was identified in the basal phenotype **(Fig. 1G)**. In the other genetic subtypes, there was no specific statistically significant association between YB-1 and DACH1 expression. These studies raised the possibilities that DACH1 may regulate the expression of YB-1 in a subset of breast cancers.

In order to determine whether a reduction of DACH1 abundance in the basal breast cancer subtype correlated independently with clinical outcome, we examined a series of basal, claudin-positive, breast cancer samples and determined the correlation with metastasis free, relapse free and overall survival. A reduction of DACH1 abundance was associated with reduced survival probability, relapse free and metastasis free survival. Similar analysis demonstrated that increased levels of YB-1 were associated with reduced metastasis free survival, relapse free survival and overall survival.

Milestone 2: <u>Determine the role of genetic-mediated breast CSC expansion in mammary tumor onset, progression and metastasis</u>.
We demonstrated that the Dach1 gene contributes to breast tumor suppression. We examined the mechanisms by identifying Dach1 interactive proteins. We demonstrated that Dach1 bound EYA and that the EYA phosphatase function was required for the induction of mammary tumorigenesis.[5] The EYA Phosphatase function contributed to the breast cancer stem cell phenotype. Phosphatases are targetable in drug screening and represent a logical next step in these studies.

We demonstrated that p53 bound Dach1. P53 has a prominent role of regulating CSC. We showed that DACH1 acetylation determined p53 binding and selectively regulated signaling by p53.[19] DACH1 co-localized with p53 in a nuclear, extranucleolar compartment and bound to p53 in human breast cancer cell lines, p53 and DACH1 bound common genes in Chip-Seq. Full inhibition of breast cancer contact-independent growth by DACH1 required p53. The p53 breast cancer mutants R248Q and R273H, evaded DACH1 binding. DACH1 phosphorylation at serine residue (S439) inhibited p53 binding and phosphorylation at p53 amino-terminal sites (S15, S20) enhanced DACH1 binding. DACH1 binding to p53 was inhibited by NAD-dependent

deacetylation via DACH1 K628. DACH1 repressed p21CIP1 and induced RAD51, an association found in basal breast cancer. DACH1 inhibits breast cancer cellular growth in an NAD and p53-dependent manner through direct protein-protein association. Functional analysis of DACH ChIP Seq and p53 ChIP Seq demonstrated several common signaling pathways, including non-small cell lung cancer (NSCLC).

Genome-wide expression analysis of mammary tumors showed DACH1 expression repressed a gene signature associated with stem cells, including Sox2, Nanog, and Klf4.[11] As SOX2 amplification is a feature of non-small cell lung cancer (NSCLC), we extended these studies to consider a role for DACH1 in NSCLC and examined the possibility that DACH1 may repress SOX2, contributing to NSCLC tumor suppression.[6] SOX2 is an amplified lineage-survival oncogene in lung squamous cell carcinoma that is oncogenic in the lung, with mutations identified in both NSCLC and SCLC. SOX2 expression is essential for lung development, can reprogram cells to pluripotency, and collaborates with FoxG1 in generating self-renewing neural precursor cells. DACH1 binds the SOX2 gene promoter in ChIP-Seq and inhibits SOX2 expression in breast cancer cells.[6] DACH1 binds FOXO binding sites, competing with endogenous FOXO proteins in ChIP assays and antagonized FOXO signaling. Thus, DACH1 inhibits 2 key pathways promoting stem cell renewal. DACH1 and SOX2 expression were inversely correlated in NSCLC but not SCLC and DACH1 repressed SOX2 via the promoter of SOX2. The DACH1-mediated repression of SOX2 was p53-dependent. SOX2 contributes to the expansion of several stem cell types. It has been proposed that a cancer stem or progenitor cell contributes to the onset of NSCLC. The repression of SOX2 by DACH1 may contribute a mechanism to inhibit NSCLC.

Milestone 3: Determine the role of secreted factors in stem cell expansion and therapy response. As we had shown a clear role for cyclin D1 in maintaining the breast and prostate stem cell, we considered the possibility that cyclin D1 may regulate secreted factors that maintain the stem cell phenotype. We showed that cyclin D1 determines the secretion of several key factors that promote cellular migration and invasion, but were unable to show a role for secreted factors in promoting stem cell expansion. Cell-conditioned medium from microRNA17/20-overexpressing noninvasive breast cancer cell MCF7 was sufficient to inhibit MDA-MB-231 cell migration and invasion through inhibiting secretion of a subset of cytokines, and suppressing plasminogen activation via inhibition of the secreted plasminogen activators (cytokeratin 8 and alpha-enolase).[14] MicroRNA17/20 directly repressed IL-8 by targeting its 3' UTR, and inhibited cytokeratin 8 via the cell cycle control protein cyclin D1. At variance with prior studies, these results demonstrated a unique mechanism of how the altered microRNA17/20 expression regulates cellular secretion and tumor microenvironment to control migration and invasion of neighboring cells in breast cancer. These findings not only reveal an anti-invasive function of miR-17/20 in breast cancer, but also identify a heterotypic secreted signal that mediates the microRNA regulation of tumor metastasis.

These studies were consistent with our previous studies in which we showed that levels of the miR-17-5p/miR-20a miRNA cluster were inversely correlated to cyclin D1 abundance in human breast tumors and cell lines. MiR-17/20 suppressed breast cancer cell proliferation and tumor colony formation by negatively regulating cyclin D1 translation via a conserved 3' untranslated region miRNA-binding site, thereby inhibiting serum-induced S phase entry. The cell cycle

effect of miR-17/20 was abrogated by cyclin D1 siRNA and in cyclin D1-deficient breast cancer cells. Mammary epithelial cell-targeted cyclin D1 expression induced miR-17-5p and miR-20a expression in vivo, and cyclin D1 bound the miR-17/20 cluster promoter regulatory region. In summary, these studies identify a novel cyclin D1/miR-17/20 regulatory feedback loop through which cyclin D1 induces miR-17-5p/miR-20a. In turn, miR-17/20 limits the proliferative function of cyclin D1, thus linking expression of a specific miRNA cluster to the regulation of oncogenesis.

We had previously proposed a model in which autophagy of the local stroma, in particular cancer associated fibrobasts, contributed "nutrients" to promote the growth of epithelial cell tumors.[15-18] We had coined the term reverse Warburg effect to describe this phenomenon. We considered the hypothesis that these micro nutrients, a product of stromal autophagy may contribute to stem cells within the tumors.

Milestone 4: Functional map of these genes and provide the identity of secreted factors in governing breast CSC expansion.
To examine this in vivo we have generated multigenic mice. The DACH1$^{-/-}$ mice are periembryonic lethal. In order to examine the role of DACH1 in mammary epithelial cell stem cell development and mammary gland development tissue specific inducible transgenic mice were developed. The multigenic mice were developed through intercrossing the Dach1$^{fl/fl}$ mice with ROSA26-Cre-ERT2 (tamoxifen inducible Cre recombinase is driven from the R26 locus). These mice were in turn inter-crossed with mice in which Cre recombinase results in the deletion of the constitutive tomato red fluorescent protein with the consequent induction of the green fluorescent protein (**Fig. 3**). Genotyping of these mice demonstrated the excision of the Dach1 allele and RT-PCR demonstrated the reduction in Dach1 expression in the mammary gland (**Fig. 3B, red circle**). The addition of tamoxifen resulted in a conversion of the mammary gland from red fluorescent to green fluorescent epithelial cells (**Fig. 3D vs. F**).

With the generation of the tri-transgenic mice we are now able to determine the role of Dach1 in mammary gland development. Based on our previous studies that DACH1 inhibits cyclin D1, and the knowledge that *cyclin D1*$^{-/-}$ mice have failed terminal alveolar breast bud development and reduced mammary stem cells, we predicted the *Dach1*$^{-/-}$ mammary gland would display increased proliferation and increased mammary gland stem cells. In our preliminary data, surprisingly, the mammary gland of the Dach1$^{fl/fl}$ upon induction of Cre with tamoxifen show reduced mammary epithelial cell proliferation (**Fig. 4**). Multiple additional mice are being examined to confirm these findings.

We identified migration stimulating factor (MSF) as a heterotypic signaling molecule that reprogrammed the myofibroblasts in the local tumor microenvironment.[16] We identified and published several other secreted factors that contribute to the heterotypic collaboration that promotes tumor progression in this model.[16,17,18] We are currently examining how these factors contribute to the expansion of cancer stem cells in the breast epithelial cell population. These studies form the basis of proposed future grants to NIH.

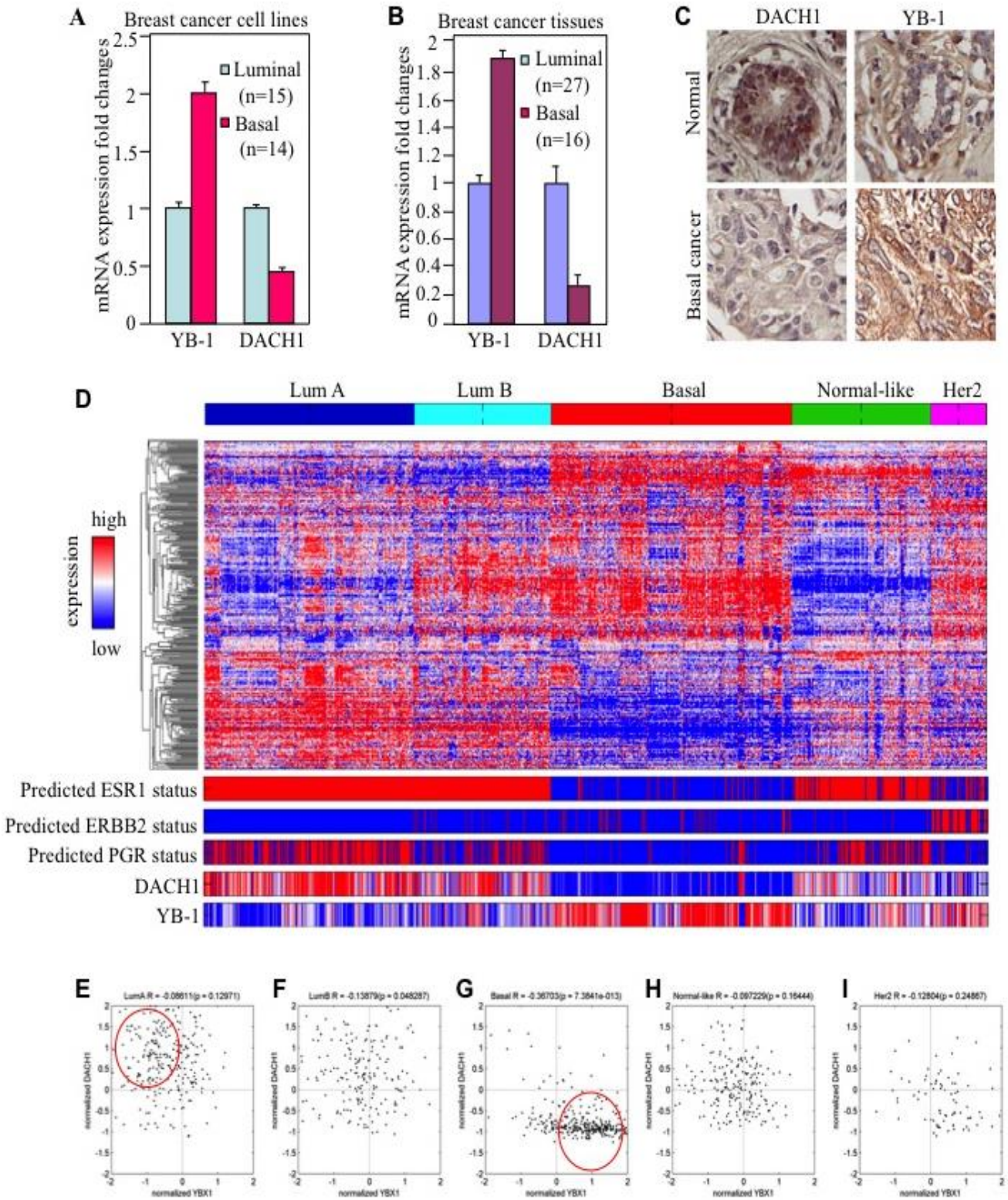

Figure 1. YB-1 and DACH1 expression is inversely correlated in human Luminal A and Basal Genetic subtypes. The mRNA abundance for YB-1 and DACH1 in (A) basal breast cancer cell lines and (B) human breast cancer cell line. C) Immunohistochemistry for DACH1 and YB-1 in normal breast and basal breast cancer (B) mRNA for YB-1 and DACH1 in A) basal like breast cancer cell lines and B) human breast cancer cell lines. D) A combined breast cancer microarray data set assigning the five breast cancer genetic expression subtypes is shown as a heat map. The ESR1, epidermal-growth factor receptor (ERBB2) and progesterone receptor (PGR), is shown together with YB-1 and DACH1 expression. E-I) Normalized DACH1 expression plotted versus normalized YB-1 expression level reveals the inverse relationship between DACH1 and YB-1 expression in luminal A and basal genetic subtype (red circle).

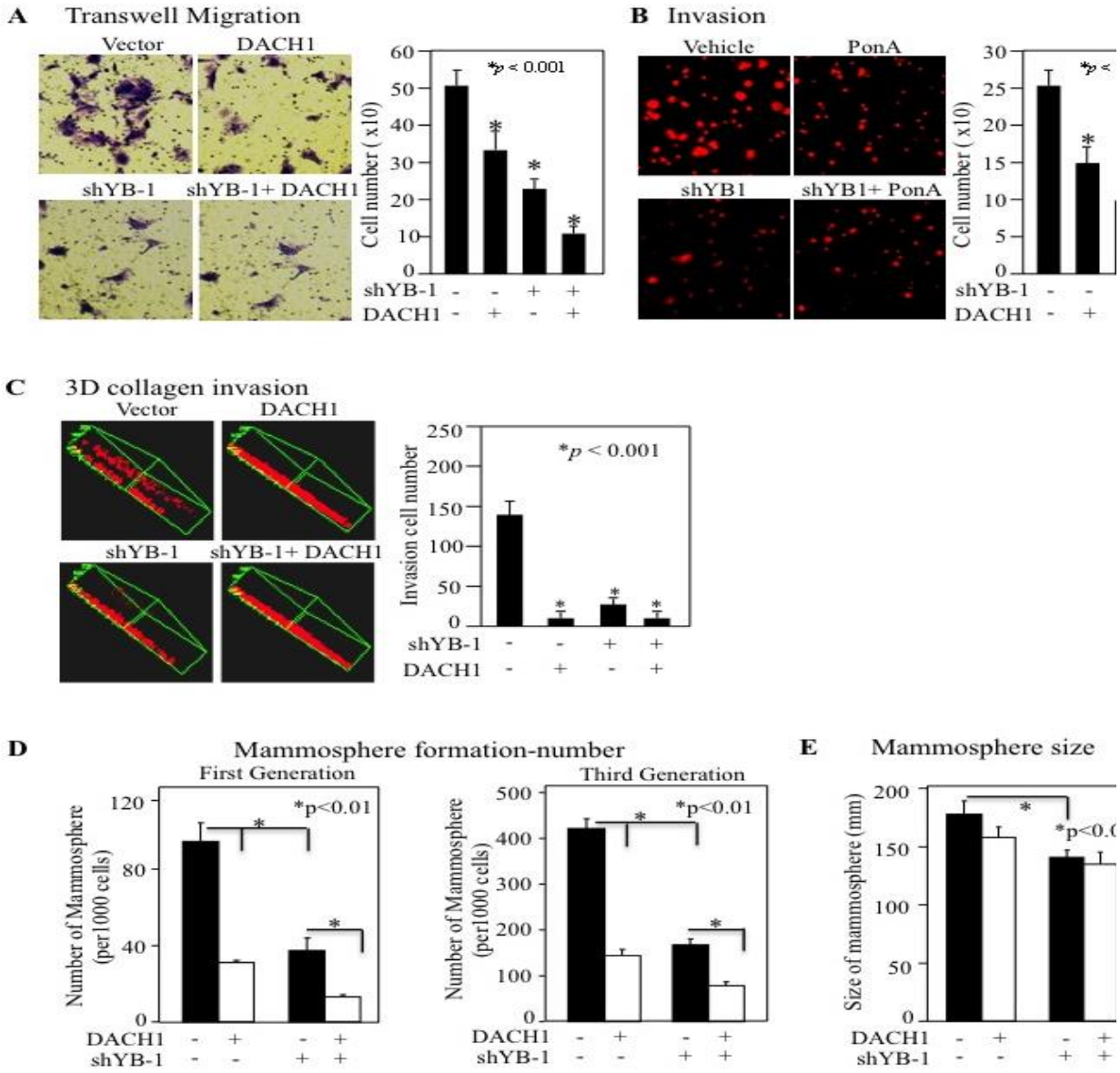

Figure 2. DACH1 antagonizes YB-1 induced migration and stem cell function. MDA-MB-231 cells, encoding Ponasterone A on a inducible DACH1, with or without YB-1 shRNA, were analyzed for migration, invasion and stem cell function using (A) transwell migration assays, (B) matrigel invasion assays and (C) 3D collagen invasion assays with the number of migrating or invading cells throughout shown as mean ±SEM for N=3 separate experiments. In (D), the same cell lines were used to determine mammosphere number and (E) mammosphere size with data shown throughout as mean ±SEM for N=5 separate experiments.

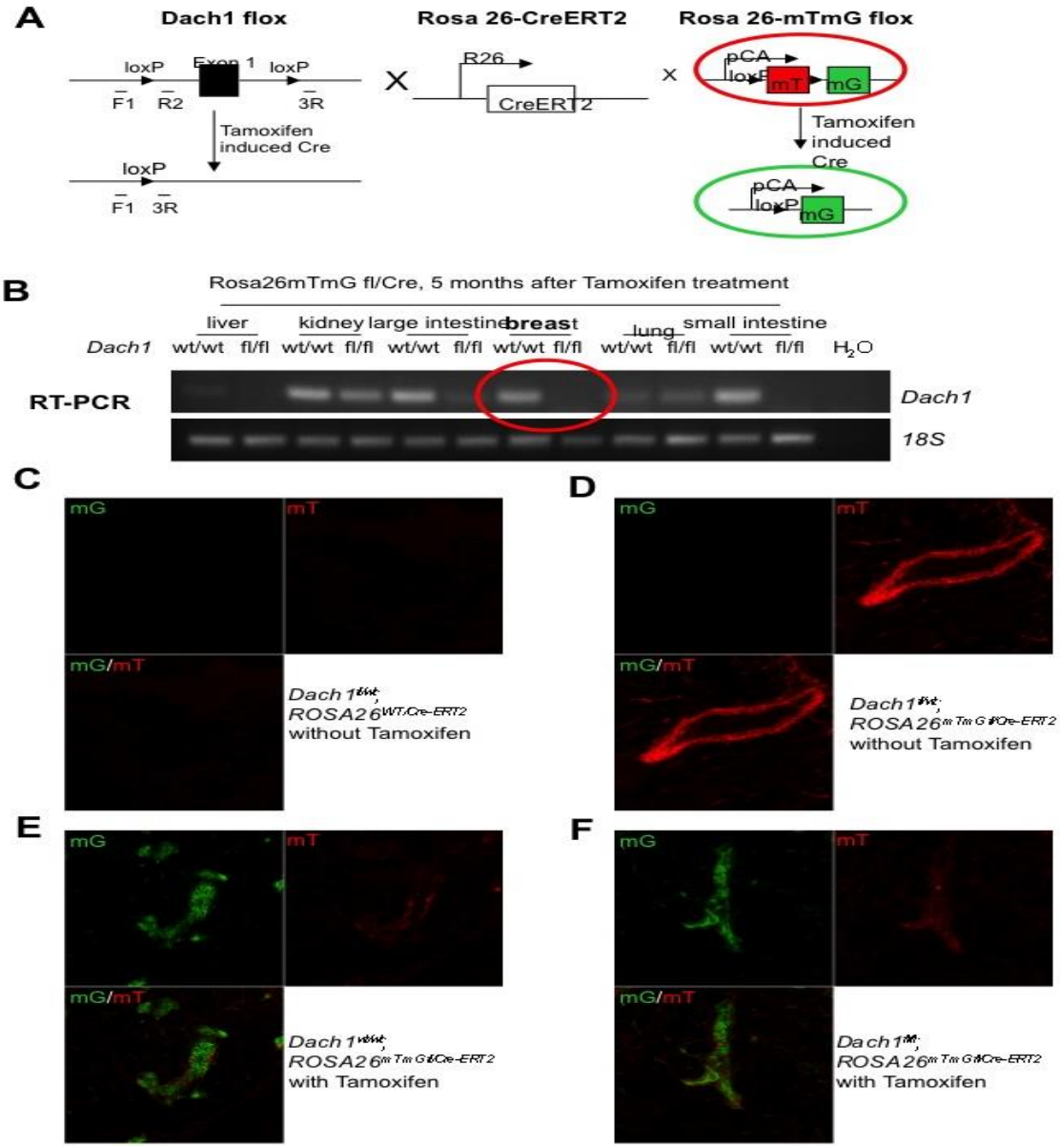

Figure 3. Analysis of Tamoxifen inducible deletion of Dach1 and RFP. (A) schematic representation of the tri-transgenic mouse lines developed to induce tomaxifen inducible deletion of the Dach1 allele. (B). RT-PCR analysis of the Dach1 in tissues as indicated. The mammary gland epitheium is highlighted with a red ring. (C-F). The relative red vs green fluorescence of the mammary gland from transgenic mice of the genotypes indicated. (D) The mammary gland of the tri-transgenic mice is constitutively expressing RFP. (F). The addition of tamoxifen in the tri-transgenic mice induces excision of Dach1 and the RFP resulting in the GFP expression in the mammary epithelium.

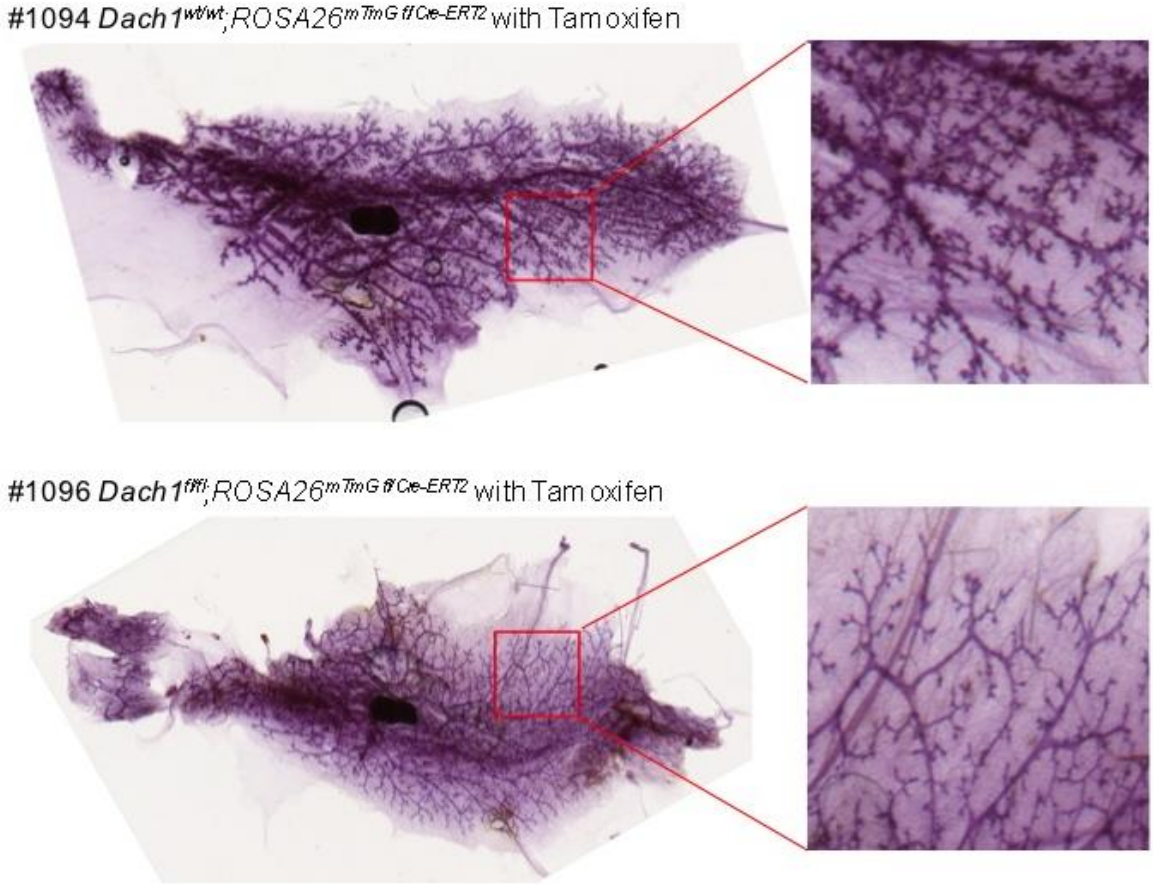

Figure 4. DACH1 enhances mammary gland terminal alveolar breast bud development and ductal branching. Mammary gland squash analysis of transgenic mice either (above) wild type for cyclin D1 or below deleted of the cyclin D1 gene in the mammary gland through the addition of tamoxifen to induce Cre expression and thereby excision of the floxed Dach1 allele.

REFERENCES

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2. 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**. Cyclin D1 induction of Dicer governs microRNA processing and expression in breast cancer. Nat Commun. 2013;4:2812. doi: 10.
3. Sun X, Jiao X, Pestell TG, Fan C, Qin S, Mirabelli E, Ren H, **Pestell RG**. MicroRNAs and cancer stem cells: the sword and the shield. Oncogene. 2013 Nov 18. doi: 10.1038/onc.2013.492.
4. Ju X, Casimiro MC, Gormley M, Meng H, Jiao X, Katiyar S, Crosariol M, Chen K, Wang M, Quong AA, Lisanti MP, Ertel A, **Pestell RG**. Identification of a cyclin d1 network in prostate cancer that antagonizes epithelial-mesenchymal restraint. Cancer Res. 2014 Jan 15;74(2):508-19.
5. Wu K, Li Z, Cai S, Tian L, Chen K, Wang J, Hu J, Sun Y, Li X, Ertel A, **Pestell RG**. Cancer Res. 2013 Jul 15;73(14):4488-99. EYA1 phosphatase function is essential to drive breast cancer cell proliferation through cyclin D1. doi: 10.1158/0008-5472.CAN-12-4078. Epub 2013 May 1.
6. 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, Gormley M, Wang C, McMahon SB, **Pestell RG**. Dachshund binds p53 to block the growth of lung adenocarcinoma cells. Cancer Res. 2013 Jun 1;73(11):3262-74. doi: 10.1158/0008-5472.CAN-12-3191. Epub 2013 Mar 14.
7. Yu Z, Pestell TG, Lisanti MP, **Pestell RG**. Cancer stem cells. Int J Biochem Cell Biol. 2012 Dec;44(12):2144-51. doi: 10.1016/j.biocel.2012.08.022. Epub 2012 Sep 2. Review.
8. Velasco-Velázquez MA, Homsi N, De La Fuente M, **Pestell RG**. Breast cancer stem cells. Int J Biochem Cell Biol. 2012 Apr;44(4):573-7. doi: 10.1016/j.biocel.2011.12.020. Epub 2012 Jan 9. Review.
9. Velasco-Velázquez MA, Popov VM, Lisanti MP, **Pestell RG**. The role of breast cancer stem cells in metastasis and therapeutic implications. Am J Pathol. 2011 Jul;179(1):2-11. doi: 10.1016/j.ajpath.2011.03.005. Epub 2011 Apr 28. Review.
10. Liu M, Sakamaki T, Casimiro MC, Willmarth NE, Quong AA, Ju X, Ojeifo J, Jiao X, Yeow WS, Katiyar S, Shirley LA, Joyce D, Lisanti MP, Albanese C, **Pestell RG**. The canonical NF-kappaB pathway governs mammary tumorigenesis in transgenic mice and tumor stem cell expansion. Cancer Res. 2010 Dec 15;70(24):10464-73. doi: 10.1158/0008-5472.CAN-10-0732.
11. Wu K, Jiao X, Li Z, Katiyar S, Casimiro MC, Yang W, Zhang Q, Willmarth NE, Chepelev I, Crosariol M, Wei Z, Hu J, Zhao K, **Pestell RG**. Cell fate determination factor Dachshund reprograms breast cancer stem cell function. J Biol Chem. 2011 Jan 21;286(3):2132-42. doi: 10.1074/jbc.M110.148395. Epub 2010 Oct 11.
12. Katiyar S, Casimiro MC, Dettin L, Ju X, Wagner EF, Tanaka H, **Pestell RG**. C-jun inhibits mammary apoptosis in vivo. Mol Biol Cell. 2010 Dec;21(23):4264-74. doi: 10.1091/mbc.E10-08-0705. Epub 2010 Oct 6.
13. 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 Mar 12;285(11):8218-26. doi: 10.1074/jbc.M110.100792. Epub 2010 Jan 6.
14. Yu Z, Willmarth NE, Zhou J, Katiyar S, Wang M, Liu Y, McCue PA, Quong AA, Lisanti MP, **Pestell RG**. microRNA 17/20 inhibits cellular invasion and tumor metastasis in breast cancer by heterotypic signaling. Proc Natl Acad Sci U S A. 2010 May 4;107(18):8231-6. doi: 10.1073/pnas.1002080107. Epub 2010 Apr 20.
15. Capparelli C, Chiavarina B, Whitaker-Menezes D, Pestell TG, **Pestell RG**, Hulit J, Andò S, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis. Cell Cycle. 2012 Oct 1;11(19):3599-610. doi: 10.4161/cc.21884. Epub 2012 Aug 30.
16. Carito V, Bonuccelli G, Martinez-Outschoorn UE, Whitaker-Menezes D, Caroleo MC, Cione E, Howell A, **Pestell RG**, Lisanti MP, Sotgia F. Metabolic remodeling of the tumor microenvironment: migration stimulating factor (MSF) reprograms myofibroblasts toward lactate production, fueling anabolic tumor growth. Cell Cycle. 2012 Sep 15;11(18):3403-14. doi: 10.4161/cc.21701. Epub 2012 Aug 23.
17. Guido C, Whitaker-Menezes D, Lin Z, **Pestell RG**, Howell A, Zimmers TA, Casimiro MC, Aquila S, Ando' S, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Mitochondrial fission induces glycolytic reprogramming in cancer-associated myofibroblasts, driving stromal lactate production, and early tumor growth. Oncotarget. 2012 Aug;3(8):798-810.
18. Guido C, Whitaker-Menezes D, Capparelli C, Balliet R, Lin Z, **Pestell RG**, Howell A, Aquila S, Andò S, Martinez-Outschoorn U, Sotgia F, Lisanti MP. Metabolic reprogramming of cancer-associated fibroblasts by TGF-β drives tumor growth: connecting TGF-β signaling with "Warburg-like" cancer metabolism and L-lactate production. Cell Cycle. 2012 Aug 15;11(16):3019-35. doi: 10.4161/cc.21384. Epub 2012 Aug 9.
19. Chen K, Wu K, Gormley M, Ertel A, Zhang W, Zhou J, Li Z, Rui H, Quong AA, McMahon S, Deng H, Lisanti MP, Wang C, **Pestell RG**. Acetylation of the Cell-Fate Factor Dachshund Determines p53 Binding and Signaling Module in Breast Cancer. Oncotarget. 2013 Jun;4(6):923-35.

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

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, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:
If the publication is not available electronically, provide 5 paper copies of the publication.

Publications are listed as they relate to the Milestones of the proposal. A subset of Aims (Aim 3 and 4 were undertaken as a collaborative research with Dr Lisanti, who was also supported from Tobacco funds as listed in the paper acknowledgements).

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. Cell Fate Factor DACH1 Represses YB-1-Mediated Oncogenic Transcription and Translation.	Wu K, Chen K, Wang C, Jiao X, Wang L, Zhou J, Wang J, Li Z, Addya S, Sorensen PH, Lisanti MP, Quong A, Ertel A, Pestell RG.	Cancer Res	Sept 2013	☐Submitted ☐Accepted ☒Published
2. 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
3. Identification of a cyclin d1 network in prostate cancer that antagonizes epithelial-mesenchymal restraint.	Ju X, Casimiro MC, Gormley M, Meng H, Jiao X, Katiyar S, Crosariol M, Chen K, Wang M, Quong AA, Lisanti MP, Ertel A, Pestell RG.	Cancer Res	May 2013	☐Submitted ☐Accepted ☒Published
4. 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, Gormley M, Wang C, McMahon SB, Pestell RG.	Cancer Res	August 2012	☐Submitted ☐Accepted ☒Published
5. Cancer stem cells.	Yu Z, Pestell TG, Lisanti MP, Pestell RG.	Int J Biochem Cell Biol	March 2012	☐Submitted ☐Accepted ☒Published
6. Breast cancer stem cells.	Velasco-Velázquez MA, Homsi N, De La Fuente M, Pestell RG.	Int J Biochem Cell Biol	Sept 2011	☐Submitted ☐Accepted ☒Published
7. The role of breast cancer stem cells in metastasis and therapeutic implications.	Velasco-Velázquez MA, Popov VM, Lisanti MP, Pestell RG.	Am J Pathol	March 2011	☐Submitted ☐Accepted ☒Published
8. The canonical NF-	Liu M, Sakamaki T,	Cancer Res	March	☐Submitted

kappaB pathway governs mammary tumorigenesis in transgenic mice and tumor stem cell expansion.	Casimiro MC, Willmarth NE, Quong AA, Ju X, Ojeifo J, Jiao X, Yeow WS, Katiyar S, Shirley LA, Joyce D, Lisanti MP, Albanese C, Pestell RG.		2010	☐Accepted ☒Published
9. Cell fate determination factor Dachshund reprograms breast cancer stem cell function.	Wu K, Jiao X, Li Z, Katiyar S, Casimiro MC, Yang W, Zhang Q, Willmarth NE, Chepelev I, Crosariol M, Wei Z, Hu J, Zhao K, Pestell RG.	J Biol Chem	May 2010	☐Submitted ☐Accepted ☒Published
10. C-jun inhibits mammary apoptosis in vivo.	Katiyar S, Casimiro MC, Dettin L, Ju X, Wagner EF, Tanaka H, Pestell RG.	Mol Biol Cell	August 2010	☐Submitted ☐Accepted ☒Published
11. CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis.	Capparelli C, Chiavarina B, Whitaker-Menezes D, Pestell TG, Pestell RG, Hulit J, Andò S, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP.	Cell Cycle	August 2012	☐Submitted ☐Accepted ☒Published
12. Metabolic remodeling of the tumor microenvironment: migration stimulating factor (MSF) reprograms myofibroblasts toward lactate production, fueling anabolic tumor growth.	Carito V, Bonuccelli G, Martinez-Outschoorn UE, Whitaker-Menezes D, Caroleo MC, Cione E, Howell A, Pestell RG, Lisanti MP, Sotgia F.	Cell Cycle	July 2012	☐Submitted ☐Accepted ☒Published
13. Mitochondrial fission induces glycolytic reprogramming in cancer-associated myofibroblasts, driving stromal lactate production, and early	Guido C, Whitaker-Menezes D, Lin Z, Pestell RG, Howell A, Zimmers TA, Casimiro MC, Aquila S, Ando' S, Martinez-Outschoorn UE, Sotgia F, Lisanti MP.	Oncotarget	July 2012	☐Submitted ☐Accepted ☒Published

tumor growth.				
14. Metabolic reprogramming of cancer-associated fibroblasts by TGF-β drives tumor growth: connecting TGF-β signaling with "Warburg-like" cancer metabolism and L-lactate production.	Guido C, Whitaker-Menezes D, Capparelli C, Balliet R, Lin Z, Pestell RG, Howell A, Aquila S, Andò S, Martinez-Outschoorn U, Sotgia F, Lisanti MP.	Cell Cycle	July 2012	☐Submitted ☐Accepted ☒Published
15. Acetylation of the Cell-Fate Factor Dachshund Determines p53 Binding and Signaling Module in Breast Cancer.	Chen K, Wu K, Gormley M, Ertel A, Zhang W, Zhou J, Li Z, Rui H, Quong AA, McMahon S, Deng H, Lisanti MP, Wang C, Pestell RG.	Oncotarget	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 ____X_____ No_____

If yes, please describe your plans:

We plan to publish additional papers and reviews related to the body of work conducted with this grant funding. Several manuscripts are currently in preparation.

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.

These studies have important implications for new breast cancer molecular diagnostics as we have shown DACH expression is selectively lost in the basal breast cancer phenotype. This could be of potential value as a diagnostic for this disease.

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.

Diagnostics. These studies have important implications for new breast cancer molecular diagnostics as we have shown DACH expression is selectively lost in the basal breast cancer phenotype. This could be of potential value as a diagnostic for this disease.

Targeted therapies for metastatic breast cancer. Our studies identifying DACH as a regulator of breast cancer metastasis via IL8 may provide an alternative approach to block human breast cancer metastasis in cases where DACH1 expression is extinguished.

Alternative treatments for breast cancer resistant to current therapies. Breast cancer inhibition. The finding that DACH blocks breast cancer stem cell expansion may have value in targeting the breast cancer stem cells that are thought to give rise to therapy resistance and metastasis.

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.

BIOGRAPHICAL SKETCH
Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME **Pestell, Richard G., M.B., B.S., M.D., Ph.D.**	POSITION TITLE **Professor and Chairman**
eRA COMMONS USER NAME (credential, e.g., agency login) **RPESTELL**	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Western Australia, Perth, Australia	MB BS (MD)	1981	Medicine
University of Melbourne, Melbourne, Australia	PhD	1991	Molecular Biology
University of Melbourne, Melbourne, Australia	MD (Thesis)	1991	Molecular Biology
Harvard Univ. Medical School, Cambridge, MA	Postdoctoral	1993	Molecular Biology
New York University, New York, NY	MBA	2011	Business Administration

A. Personal Statement

My research has focused on abnormalities of cell-cycle control and ways of treating cancer based on these pathways. My lab is attempting to understand the functions of cyclin D1 and the cell cycle proteins that contribute to the onset and progression of mammary tumorigenesis. We have characterized molecular genetic determinants of stem cell expansion using multigenic inducible mouse models (c-jun, p21, NFκB, DACH1, cyclin D1). Determining the role of cancer stem cells in tumor onset and progression may define novel tractable therapeutic targets. I have successfully administered R01 and P30 Projects (e.g. staffing, research protections, budget); collaborated with other researchers and produced ~500 peer-reviewed publications. I have mentored students for >18 years, many of which have obtained academic positions. In summary, I have a demonstrated record of successful and productive research projects in an area of high relevance, and my expertise and experience have prepared me to assist in this proposed project.

B. Positions and Honors

<u>Positions and Employment</u>

1982-1991	Intern, Registrar, (Endocrin. and Oncol.) Royal Perth & St. Vincent's Hospital, Melbourne; NHMRC Scholar, University of Melbourne, (Dept. of Medicine), Australia
1991-1993	Clin. & Res. Fellow, Mass General Hospital, Harvard Univ. Medical School, Cambridge, MA
1993-1996	Assistant Prof., Dept. of Med. Northwestern Univ. Med. School, Chicago, IL.
1996-2001	Associate Prof. and Prof., Dept. of Med., Developmental and Mol. Biol., Albert Einstein College of Medicine (AECOM), Yeshiva University, New York, NY.

2000-2002	Chairman, Division of Hormone-Dependent Tumor Biology & Director, Program in Hormone Dependent Cancers, AECOM
2002-11/2005	Director, Lombardi Comprehensive Cancer Center, Associate Vice President, Georgetown University Medical Center, Chairman, Dept. of Oncology, Professor of Medicine, Georgetown University, Washington, DC
12/2005-Present	Director of the Kimmel Cancer Center, Vice President of Oncology Services, Associate Dean of Cancer Programs, Chairman of the Dept. of Cancer Biology, Thomas Jefferson University Hospitals, Philadelphia, PA
07/2008-04/2010	Founding Director, Delaware Valley Institute for Clinical & Translational Science
07/2008-06/2013	Honorary Professor, Department of Medicine, University of Melbourne, Melbourne, Australia

Honors

University Commonwealth Scholarship (1975), Sandoz Medical Research Prize (1975), NHMRC Postgraduate and HECS Scholarships (1988), Winthrop Travelling Fellowship (1990), Fellow of Royal Australian College of Physicians (1991), Australian Genome Conference Prize (1991), Neil Hamilton Fairley NHMRC Fellowship (1996), NIH FIRST Award (1996), NIH Shannon Award (1996), Irma T. Hirschl Weil Caulier Career Award (1998), Pfeiffer Award (1998), Chair of Susan Komen Study Section (1999), Member of American Society Clinical Investigators (2000), Gragnani Endowed Chair (2002), Endocrine Society of Australia's Harrison Award (2005), Fellow of Royal Society of Medicine (2007), Doctoris Honoris Causa (UWA) (2008), Susan Komen Light of Life Award (2010), Fellow, American Association for the Advancement of Science (2011)

C. Selected Peer-Reviewed Publications (Selected from >615 published works with ~34,000 citations)

1. Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell RG, Ben-Ze'ev A. *The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway.* Proc Natl Acad Sci U S A. 1999 May 11; 96(10): p. 5522-7.
2. Amanatullah DF, Reutens AT, Zafonte BT, Fu M, Mani S, Pestell RG. *Cell-cycle dysregulation and the molecular mechanisms of prostate cancer.* Front Biosci. 2000 Apr 1; 5: p. D372-90.
3. Fu M, Rao M, Wang C, Sakamaki T, Wang J, Di Vizio D, Zhang X, Albanese C, Balk S, Chang C, Fan S, Rosen E, Palvimo JJ, Janne OA, Muratoglu S, Avantaggiati ML, Pestell RG. *Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth.* Mol Cell Biol. 2003 Dec; 23(23): p. 8563-75.
4. Williams TM, Hassan GS, Li J, Cohen AW, Medina F, Frank PG, Pestell RG, Di Vizio D, Loda M, Lisanti MP. *Caveolin-1 promotes tumor progression in an autochthonous mouse model of prostate cancer: genetic ablation of Cav-1 delays advanced prostate tumor development in tramp mice.* J Biol Chem. 2005 Jul 1; 280(26): p. 25134-45.
5. Xiao D, Chinnappan D, Pestell RG, Albanese C, Weber HC. *Bombesin regulates cyclin D1 expression through the early growth response protein Egr-1 in prostate cancer cells.* Cancer Res. 2005 Nov 1; 65(21): p. 9934-42.
6. Gong J, Zhu J, Goodman OB Jr., Pestell RG, Schlegel PN, Nanus DM, Shen R. *Activation of p300 histone acetyltransferase activity and acetylation of the androgen receptor by bombesin in prostate cancer cells.* Oncogene. 2006 Mar 30; 25(14): p. 2011-21.
7. Casimiro M, Rodriguez O, Pootrakul L, Aventian M, Lushina N, Cromelin C, Ferzli G, Johnson K, Fricke S, Diba F, Kallakury B, Ohanyerenwa C, Chen M, Ostrowski M, Hung MC, Rabbani SA, Datar R, Cote R, Pestell RG, Albanese C. *ErbB-2 induces the cyclin D1 gene in prostate epithelial cells in vitro and in vivo.*

Cancer Res. 2007 May 1; 67(9): p. 4364-72.

8. Di Vizio D, Sotgia F, Williams TM, Hassan GS, Capozza F, Frank PG, Pestell RG, Loda M, Freeman MR, Lisanti MP. *Caveolin-1 is required for the upregulation of fatty acid synthase (FASN), a tumor promoter, during prostate cancer progression.* Cancer Biol Ther. 2007 Aug; 6(8): p. 1263-8.
9. Wu K, Katiyar S, Witkiewicz A, Li A, McCue P, Song LN, Tian L, Jin M, Pestell RG. *The cell fate determination factor dachshund inhibits androgen receptor signaling and prostate cancer cellular growth.* Cancer Res. 2009 Apr 15; 69(8): p. 3347-55.
10. Di Vizio D, Morello M, Sotgia F, Pestell RG, Freeman MR, Lisanti MP. *An absence of stromal caveolin-1 is associated with advanced prostate cancer, metastatic disease and epithelial Akt activation.* Cell Cycle. 2009 Aug; 8(15): p. 2420-4.
11. Gravina GL, Marampon F, Di Staso M, Bonfili P, Vitturini A, Jannini EA, Pestell RG, Tombolini V, Festuccia C. *5-azacitidine restores and amplifies the bicalutamide response on preclinical models of androgen receptor expressing or deficient prostate tumors.* Prostate. 2010 Aug;70(11):1166-78. [Epub 2010 Mar 23].
12. Vissapragada S, Ghosh A, Ringer L, Salinas P, Brophy A, Peaceman D, Kallakury B, Banerjee PP, Fricke ST, Helfrich W, Lee YC, Pestell RG, Scherer P, Tanowitz HB, Avantaggiati ML, Hilakivi-Clarke L, Lisanti MP, Rodriguez OC, Albanese C. *Dietary n-3 polyunsaturated fatty acids fail to reduce prostate tumorigenesis in the PB-ErbB-2 x Pten(+/-) preclinical mouse model.* Cell Cycle. 2010 May 15; 9(9).
13. Powell MJ, Casimiro MC, Cordon-Cardo C, He X, Yeow WS, Wang C, McCue P, McBurney MW, Pestell RG. *Disruption of a Sirt1 Dependent Autophagy Checkpoint in the Prostate Results in Prostatic Intraepithelial Neoplasia Lesion Formation.* Cancer Res. 2011 Feb 1;71(3):964-75. Epub 2010 Dec 28.
14. Gravina GL, Marampon F, Piccolella M, Motta M, Ventura L, Pomante R, Popov VM, Zani BM, Pestell RG, Tombolini V, Jannini E, Festuccia C. *Hormonal therapy promotes hormone-resistant phenotype by increasing DNMT activity and expression in prostate cancer models.* Endo. 2011 Dec;152(12):4550-61. Epub 2011 Oct 11.
15. Ju X, Ertel A, Casimiro C, Yu Z, Meng H, McCue P, Walters R, Fortina P, Lisanti M, Pestell RG. *Novel Oncogene Inducted Metastatic Prostate Cancer Cell Lines Define Human Prostate Cancer Progression Signatures.* Cancer Res. 2013 Jan 15;73(2):978-89. Epub 2012 Nov 30.

D. Research Support

Ongoing Research Support

NIH P30 CA 056036-14 Pestell (PI) 06/22/95-05/31/18
Cancer Center Support Grant – Translational Research in Cancer
Specific Aim: This proposal for a Cancer Center Support Grant (CCSG) requests funding to support the cancer research activities of the Kimmel Cancer Center (KCC) in order to increase the survival and quality of life of cancer patients by translating basic research discoveries into new strategies to prevent, diagnose, monitor and cure human cancer.

American Cancer Society Pestell (PI) 01/01/08-7/31/15
Institutional Research Grant

NIH R01 CA086072-12 Pestell (PI) 3/1/09-02/28/14
Androgen Receptor Function in Prostate Cancer

Specific Aim: Determine the role of cyclin D1 and androgen receptor mutations in prostate cancer cellular growth.

NIH R01 CA137494 Pestell/Knudsen (PI) 08/01/10-01/31/15
Impact of Cyclin D1 Isoforms in Breast Cancer
Specific Aims: 1) Determining the mechanisms through which cyclin D1 proteins control cell cycle progression and the response to specific anti-mitogenic stresses. 2) Defining the impact of cyclin D1a/D1b on pathological features of breast cancer progression. 3) Delineating the function of these proteins in relation mammary tumorigenesis and disease outcome.

Breast Cancer Research Foundation Pestell (PI) 10/01/11-09/30/14
Genetic Targets of Breast Tumor-Initiating Cells
Specific Aim: Determine the role of key genetic targets in breast tumor stem cell expansion.

Falk Trust Pestell (PI) 07/01/06-08/30/14
The Role of MicroRNA Gene Expression in Human Breast Cancer
Specific Aims: 1) Define the genetic determinants of breast tumor stem cell expansion. 2) Determine the role of genetic mediated breast tumor stem cell expansion in mammary tumor onset, progression and metastasis. 3) Determine the role of secreted factors in stem cell expansion and therapy response.

Completed Research Support

NIH R01 CA132115-04 Pestell (PI) 07/20/09-06/30/13
DACH1/Eya Cell-fate Determination Factor and Mammary Tumorigenesis
Specific Aims: 1) Determine the mechanisms governing DACH1/Eya inhibition of breast tumor cellular proliferation. 2) Determine the role of DACH1 as a suppressor of mammary tumor onset, progression and metastasis using *Dach1*$^{f1/f1}$ transgenic mice. 3) Determine the mechanism by which DACH1/Eya inhibits cellular migration.