# Final Progress Report for Research Projects Funded by Health Research Grants

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

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

2. **Reporting Period (start and end date of grant award period):** 01/01/2009-12/31/2012

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

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

5. **Grant SAP Number:** 4100047652

6. **Project Number and Title of Research Project:** 5 -The Role of MicroRNA (miRNA) Gene Expression in Therapy Resistance of Human Breast Cancer

7. **Start and End Date of Research Project:** 1/1/2009 - 06/30/2012

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

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:

   $ $660,950.56

   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** | **Position Title** | **% of Effort on Project** | **Cost** |
|---|---|---|---|
| Pestell, R. | Principal Investigator | 2% Years 1 & 2 | $7,652 |
| Tian, L | Research Instructor | 100% Yrs.1 & 2 | 64,866.92 |
| Chen, L | Postdoc Fellow | 100% Yrs. 1&2 | 34,428.60 |
| Popov, V | Postdoc Fellow | 50% Yr. 2 | 8,574.62 |
| Li, Z | Assistant Professor | 25% Yr. 3 | 8,937.72 |
| Hasan, R | Research Assistant | 100% Yrs. 1&2 | 12,136.91 |
| Velasco, V | Research Instructor | 100% Yr.2 | 25,226.10 |
| Loro, E | Postdoc Fellow | 100% Yr.2 | 12,061.42 |
| DiSante, G | Postdoc Fellow | 100% Yr.3 | 32,727.51 |
| Bisetto, S | Research Tech | 100% Yr.3 | 27,749.07 |
| Markley, K | Research Assistant | 5% Yr.01 | 273.74 |
| Brodzik, R | Research Asscociate | 100% Yrs. 2&3 | 65,501.76 |

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: |
|---|---|---|---|---|
| R01CA070896-Regulation of cyclin D1 Expression | ☒NIH<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify:_) | July 2012 | $2,555,759 | $<br>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:

Resubmission of NIH R01CA070896

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

Submit R01 grant and obtain funding

**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_____ No____X_____

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

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | |
| Female | | | | |
| Unknown | | | | |
| **Total** | | | | |

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

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

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

Yes____X____ No_____

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

| **Name & Degree (s)** | **Previous Affiliation** |
|---|---|
| Lifeng Tian, Ph.D. | National Center of Biomedical Analysis, China |
| Vladimir Popov, Ph.D. | Ramapo College of New Jersey, New Jersey |
| Zhiping Li, M.D., Ph.D. | Georgetown University, Washington, D.C. |
| Marco Velasco-Velázquez, Ph.D. | National Autonomous University of Mexico, Mexico |
| Emanuele Loro, Ph.D. | University of Padova, Italy |
| Gabriele DiSante, Ph.D. | University of L'Aquila, Italy |
| Sara Bisetto, M.S. | University of Padova, Italy |
| Robert Brodzik, Ph.D. | Polish Academy of Sciences, Poland |

**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.

miRNA data sets signature / miRNA expression systems / generator

**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.**

These studies have resulted in 1 submitted publication, 6 published papers, and 3 manuscripts in preparation.

The key findings in this grant are that:

Aim 1. Isolate the cellular intermediates in the progression from the primary breast tumor to the metastatic tumor and determine the changes in miRNA gene expression that accompany this progression.

microRNA 17/20 inhibits cellular invasion and tumor metastasis in breast cancer by heterotypic signaling. In these studies we examined the miRNA expression in the breast tumors of patients that had either spread to the lymph nodes, or patients in which the breast cancer had not spread to the lymph nodes. We found that the abundance of the miRNA 17/20 cluster in the primary tumor correlated inversely with metastatic risk. The microRNA17/20 cluster was shown to govern cellular migration and invasion of nearby cells via heterotypic secreted signals. microRNA17/20 abundance was reduced in highly invasive breast cancer cell lines and node-positive breast cancer specimens. 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). 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 published in the Proceedings of the National Academy of Sciences as:

> 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 USA. 2010 May 4;107(18):8231-6. Epub 2010 Apr 20.

Aim 2. Elucidate the changes in miRNA expression in breast cancer associated with the development of resistance to tamoxifen and chemotherapeutic agents therapy.

We conducted several parallel types of experiments.

Hierarchical clustering of >400 human breast cancer defines 4 miRNA subtypes. In order to define the miRNA expression in breast cancer associated with response to therapy we developed the first superset of breast cancer miRNA. This superset of >400 patient samples was used to define by hierarchical clustering 4 distinct subtypes of breast cancer based on miRNA (Fig. 1). The superset segregated by relatedness through hierarchical clustering into 4 distinct groups (G1 – G4). The predicted mRNA genetic subtype of each patient was designated and aligned with the miRNA subtype (Fig. 1A). The miRNA subtype did not correlate significantly with the mRNA genetic subtype (Basal, luminal A, luminal B, Normal Her 2 positive, normal like). The miRNA subtypes did however correlate with distinct distal relapse free survival. The G2 subtypes correlated with better outcome and G4 correlated with poor outcome (Fig. 1B). The miRNA subtype correlated significantly with outcome with a Z score of 2.7 ($p<0.007$) (Fig. 1C). The subtypes were compared to G1 for difference in outcome of distant, relapse free survival. The hazard ratio of G3 and G4 vs. G2 were 1.87 and 2.07 ($p=0.08$ and $p=0.02$) respectively.

The cyclin D1-regulated miRNA signature correlated with ERα positive tumors and better outcome breast cancer. In order to determine the relationship of miRNA status to tamoxifen response we next examined the ERα status of each miRNA subtype of the patients outcome of patients based on their miRNA signature. The ERα status by miRNA subtype is shown in Figure 1A.

Previous studies had shown that cyclin D1 overexpression induced tamoxifen resistance. We therefore defined a cyclin D1 induced miRNA signature in MCF7 cells. Quantitative real-time RT-PCR based miRNA analysis was conducted comparing the miRNA expression profiles in MCF-7 cells, or MCF-7 cells treated with cyclin D1 siRNA. Among 742 examined miRNAs, ~500 were detectable with ~50% showing >1.5-fold reduced expression in cyclin D1 knock-down cells. miR-21, the most abundant miRNA in MCF-7 cells was not regulated by endogenous cyclin D1. The cancer-related miR-17-92 cluster and its paralogs were induced by cyclin D1. miRNA of oncomir clusters included miR-17-20, the C19MC cluster (miR-513a or 518f, 519d, 520b, 524, 506) and miR-221.

We then used the cyclin D1 miRNA signature to determine if there was a relationship with the ERα status (ERα is necessary for a tamoxifen response). The cyclin D1 induced miRNA signature correlated closely with the ERα positive tumors. We next compared patients with ERα positive vs. ERα negative breast tumors. Hierarchical clustering identified enrichment for the cyclin D1 miRNA signature with ERα positive status. We next segregated patient tumors by grade and determined whether the cyclin D1 miRNA signature correlated with tumor grade. The cyclin D1 miRNA signature was associated with low grade tumors defined by histology. In order to determine whether the cyclin D1 miRNA signature correlated with a corresponding miRNA cluster subtype, we aligned the cyclin D1-regulated miRNA signature with the previously defined miRNA subtypes of breast cancer. Cyclin D1 miRNA were enriched in the G2 miRNA subtype.

Together, these studies demonstrate that the cyclin D1-regulated miRNA signature correlates with ERα positive tumors, tumor grade and better prognosis. These findings are consistent with our previous published studies of 2250 patients in which enrichment of the cyclin D1 mRNA signature was found with the luminal A subtype (good prognosis) and anti-correlation with basal (bad prognosis).

The manuscript synopsis is:

C*yclin D1* encodes the regulatory subunit of a holoenzyme that phosphorylates the pRB protein and promotes $G_1$/S cell cycle progression and oncogenesis. The mechanism by which the cell cycle directly controls the non-coding genome is poorly understood. Herein, a superset of miRNA were generated from >400 patient breast cancer samples with recurrence free survival outcome. Hierarchical clustering of the breast cancer miRNA superset defined four distinct miRNA clusters (G1-4). The miRNA clusters were associated with distinguishable relapse-free survival (p=0.007). The G4 cluster associated with poor relapse free survival and G2 with improved outcome. shRNA-mediated reduction in cyclin D1 abundance was used to determine the miRNA signature maintained by endogenous cyclin D1 in human breast cancer cells. A signature of cyclin D1-regulated miRNA, either repressed or induced by cyclin D1, was identified. The cyclin D1-mediated miRNA signature, was enriched in the G4 miRNA cluster, correlated with ERα positive, low grade tumors and was associated with improved relapse-free survival and improved outcome. KEGG analysis of the cyclin D1-regulated miRNA cluster demonstrated enrichment for the Wnt/*β*-catenin pathway. The cyclin D1 miRNA signature had seed elements in 25 genes of the Wnt signaling pathway in vivo studies using inducible transgenics demonstrated cyclin D1. Cyclin D1 orchestrates a miRNA signature that is embedded in a subtype of human breast cancer associated with Wnt/*β*-catenin.

These studies form the basis of a publication currently in preparation entitled:

> Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. *Cyclin D1-Mediated MicroRNA Expression Signature in Breast Cancer Subtype and Outcome*. (In Preparation).

<u>miRNA in chemotherapeutic response</u>. We identified miRNA 17/20 as specifically contributing to tamoxifen and DNA damaging agent induced apoptosis.

The cyclin D1 regulated miRNA 17/20 sensitizes cells to apoptosis via Akt1. We had previously shown that cyclin D1 regulates miRNA 17/20 via binding to the regulatory region of the miRNA 17/20 cluster. We further examined the significance of miRNA 17/20 in breast cancer cells by stably expressing miRNA 17/20 in MCF-7 cells. DNA damaging agents (UV irradiation, doxorubicin) and tamoxifen showed enhanced apoptosis in the miRNA 17/20 overexpressing MCF7 cells. antimiRNA to miRNA 17/20 reduced DNA damaging agent-induced apoptosis (Fig. 2). Akt1 was required for the miRNA 17/20 enhancement of apoptosis as miRNA 17-20 induced apoptosis was abrogated in Akt1 knockout cells. Together these studies suggest the role for cyclin D1 regulated miRNA 17/20 in tamoxifen- and chemotherapy- induced apoptosis.

The results of these studies are currently in a manuscript entitled:

> Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. *mir17-20 sensitizes breast cancer cells to tamoxifen-mediated apoptosis, via AKT1*. (In Preparation).

Aim 3. Elucidate the miRNA signatures regulating breast cancer stem cells.

Cyclin D1 induces miRNA processing via induction of Dicer abundance. Compelling evidence supports a model in which Dicer abundance contributes to the processing of miRNA and in the development of stem cells. Dicer-deficient mice are defective in the production of stem cells. Dicer mediated upregulation of BCRP confers tamoxifen resistance. We therefore conducted studies to examine the mechanism governing Dicer abundance in human breast cancer. These studies demonstrated that Dicer expression is induced by cyclin D1 via transcription of the Dicer promoter. In our analysis of >2250 breast cancer samples, a correlation between Dicer and cyclin D1 abundance was found in luminal A and basal breast cancer subtypes (Fig. 3). These studies form the basis of a manuscript currently in Review at EMBO Journal and were outlined as part of the prior annual report.

Synopsis: Cyclin D1 induces miRNA biogenesis through the induction of the processing enzyme Dicer.

C*yclin D1* encodes the regulatory subunit of a holoenzyme that phosphorylates the pRB protein and promotes $G_1$/S cell cycle progression and oncogenesis. *Dicer* is a central regulator of miRNA maturation, encoding an enzyme that cleaves double strand RNA or stem-loop-stem RNA into 20-25 nucleotide long small RNA governing sequence specific gene silencing and heterochromatin methylation. The mechanism by which the cell cycle directly controls the non-coding genome is poorly understood. Herein, *cyclin D1*$^{-/-}$ cells showed reduced miRNA expression and defective miRNA processing both of which were restored by cyclin D1a overexpression. Cyclin D1a induced Dicer expression *in vitro* and *in vivo* through direct transcriptional targeting of Dicer, via a cdk-independent mechanism. Dicer and cyclin D1a induced heterochromatic histone modification (TriM-H3K9), independently of pRB. Cyclin D1a-dependent cellular proliferation and cellular migration was Dicer-dependent. A positive correlation between cyclin D1a and Dicer expression in luminal A and basal subtypes of human

breast cancer is related with 10-year survival rate of patients. These findings demonstrated cyclin D1a induction of Dicer expression and coordination of miRNA biogenesis, correlating with cellular proliferation and migration of cancer cells and long-term survival of cancer patients in human breast cancer.

The results of these studies are currently in a manuscript entitled:

> Yu Z, Wang L, Ju X, Wang M, Ertel A, Loro E, Li Z, Wu K, Gormley M, Wang C, Fortina P, Tozeren A, Pestell RG. *Cyclin D1 Induction of Dicer Governs MicroRNA processing and Expression in Breast Cancer*. EMBO J. (In review).

<u>Cyclin D1 enhances murine stem cell expansion via heterotypic signals.</u> In our investigation of the mechanism governing breast cancer stem cell expansion, we observed that the mammary stem cell population induced by pregnancy was reduced in the *cyclin D1*$^{-/-}$ mammary gland (Fig. 4). We conducted analysis of the mammary gland of *cyclin D1*$^{-/-}$ vs cyclin D1 wt mice. Analysis of the mammary epithelial stem cells in preganancy demonstrated a reduction in the proportion of CD24$^{-}$CD2q$^{-}$lin$^{-}$ cells indicative of a population of mammary stem cells.

We sought to determine the molecular mechanisms by which cyclin D1 induced stem cell expansion. We considered the possibility that cyclin D1 may mediate stem cell expansion via cell autonomous or via non-cell autonomous mechanisms. In order to test this hypothesis, we conducted co-culture experiments in which fibroblasts derived from wt or *cyclin D1*$^{-/-}$ mice were co- cultured with murine embryonic stem cells. The expansion of mES cells by co culture was reduced in the *cyclin D1*$^{-/-}$ cells. We generated *cyclin D1*$^{-/-}$/*Rb*$^{-/-}$ mice and *Rb*$^{-/-}$ mice and compared the capacity of MEFs to support mES cell expansion. The *cyclin D1*$^{-/-}$ MEFs showed reduced ability to support mES cell expansion (Fig. 5). We conducted an analysis of the components secreted by *cyclin D1*$^{-/-}$ MEFs and identified stem cell factor (SCF) as reduced in the *cyclin D1*$^{-/-}$ MEF supernatant. The *cyclin D1*$^{-/-}$ MEFs also regulated other forms of stem cells via heterotypic secreted signals including the hematopoietic stem cells. In this regard cyclin D1 enhanced the formation of hematopoietic stem cells (HSC) defined by CD34+ cells. We are currently testing SCF as the distal mediator of cyclin D1-mediated HSC expansion. We propose MiR-17/20/93/106 which are induced by cyclin D1 may contribute to the phenotype via SQSTM1.

Approximately 150 CTC samples have been collected. With the miRNA hierarchical clustering conducted on >400 human breast cancers, we have an architecture for comparison with the breast CTC data. We now have defined miRNA breast cancer subtypes and their correlation with ERα status and outcome, therefore we will be able interpret the breast CTC data.

Methods and Materials

siRNAs and transfection. All siRNAs were from Qiagen. Target sequence for cyclin D1: AACAAGCTCAAGTGGAACCTG; Target sequence for negative control siRNA: AATTCTCCGAACGTGTCACGT; Lipofectamine RNAiMax (Invitrogen) was used the reverse transfect siRNA to MCF-7 cells following the manufacturer's instructions.

Small RNA library preparation and deep sequencing. Small RNA-enriched RNA was prepared using mirVana™ miRNA Isolation Kit from Ambion. The small RNA fractions (10-50 nt) were purified using the flashPAGE™ Fractionation (Ambion). Small RNA libraries were prepared using the SOLiD™ Small RNA Expression Kit (Applied Biosystems) following the manufacturer's instructions. cDNA libraries were amplified by standard emulsion PCR using primers complementary to adaptors. After amplification, the library was sequenced by the SOLiD™ 4 System (Applied Biosystems).

Mapping of the reads. Reads were preprocessed using "cutadapt" and mapped on the human genome using BWA. During mapping base quality values were taken into account and no more than 2 mismatches (but no insertions or deletions) were allowed. Reads that mapped at multiple locations were discarded and not considered further. All miRNA reads were normalized with a housekeeping small RNA SNORD44. Only those miRNAs with reads pileup value more than 25 and fold-change more than 1.5 were considered as differentially expression. The miRNA reference was based on the miRBase 17 database (http://www.mirbase.org/).

miRNA analysis was performed using the Rodent OneArray® miRNA v5 (Phalanx Biotech, Belmont, CA). RNA quality and integrity were determined utilizing an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and absorbance at A260, A280 and A230. RNA, having a RIN of >5.0, A260/280 absorbance ratio of >1.6, and an A260/230 absorbance ratio of >1 was utilized for further experimentation. RNA was enriched using a 2-step process with Nanosep 100k (Pall, Port Washington, NY, USA) and Vivaspin 500 3k filters (Sartorius, Göttingen, Germany). Small RNA was labeled with Cy5-ULSTM (Kreatech Diagnostics, Amsterdam, Netherlands). Labeled RNA was hybridized at 37°C overnight using miRNA OneArray® Hybridization Chamber with 1X OneArray Hybridization Buffer I and II (Phalanx Biotech). After hybridization, the arrays were washed according to the OneArray protocol. Raw intensity signals for each miRNA array were captured using a Molecular Dynamics™ Axon 4100A scanner, measured using GenePixProTM Software, and stored in GPR format. The data from all miRNA arrays in each experimental set was then passed to R: Limma, RColorBrewer and Gene filter for combining technical replicates and performing statistical analyses.

miRNA expression profiling and data analysis. miRNA expression profile analysis in MCF-7 cells was performed using microRNA Ready-to-Use PCR, Human Panel I+II, V2.M (Product no:203607) and miRCURY LNA™ Universal cDNA Synthesis Kit (Product no:203300) from Exiqon following the Manufacturer's Instructions. Inter-plate calibration and reference small RNAs for normalization was applied to analyze the data following the Data Analysis Guide (Exiqon). The experiments were repeated in two sets of separate samples, and only the miRNAs differentially expressed more than 1.5-fold change (control siRNA *vs. cyclin D1* siRNA) in both sets of samples were chosen for further analysis.

Analysis of transcript co-expression within public microarray. Gene expression profiles were median-centered, scaled to unitary standard deviation, and 2D-scatter plots were produced for Dicer versus *cyclin D1* transcript expression in the luminal A subtype of breast cancer. The number of samples bound in each of the four quadrants around x=0 and y=0 was used to build a contingency table. The observed number of samples in each the four quadrants was assessed for significance by comparing against the expected distribution – the total number of samples in each quadrant, using a Chi-square test.

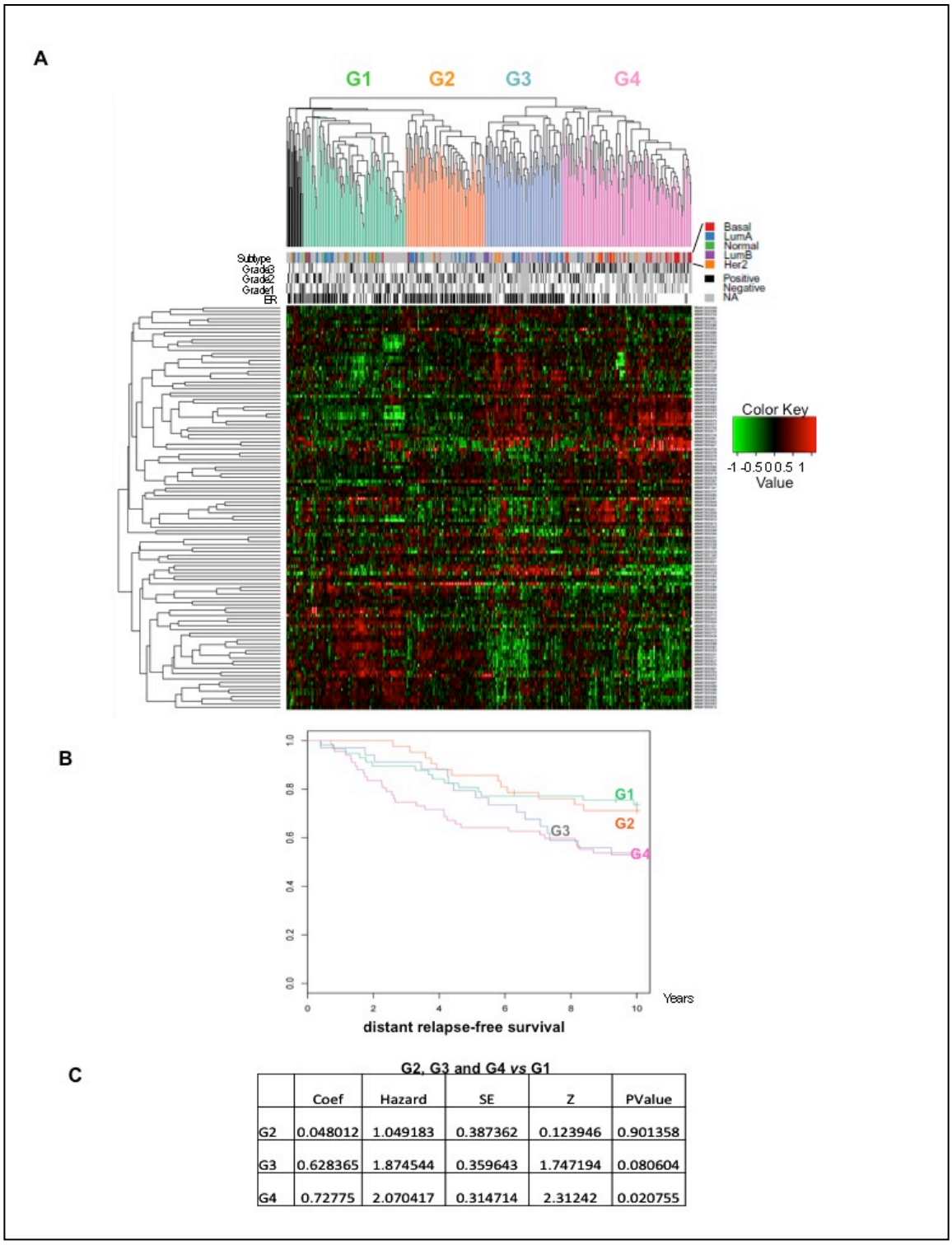
Identification of clinically relevant miRNA. Statistical hypothesis testing was used to identify miRNA expression profiles associated with clinically relevant covariates and survival in breast cancer. Differential expression of miRNAs was detected with the limma Bioconductor package, using the Benjamini-Hochberg approach was to calculate the false discovery rate. Significant miRNA expression profiles were identified with q-values $\leq 0.05$. For covariates with multiple classes (e.g. molecular subtype), data was arranged to test each class versus all others. Association of miRNA expression with relapse-free survival was tested using Kaplan-Meier analysis.

Western blot analysis. The following antibodies were used for western blotting: TSP ab-11 was from Neomarker (Fremont, CA); anti-cyclin D1 (sc-20044), anti-Rb (sc-7905), anti- β-actin (sc-47778), anti-β-tubulin (sc-9104) were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-OPN (ab8448) and anti-Socs1 (ab62584) were from Abcam (Cambridge, MA).

Mice. All animal experiments were done in accordance with the guidelines for the care and use of laboratory animals at Thomas Jefferson University. *Cyclin D1*$^{-/-}$ mice were maintained on a mixed C57B1/6x129/SvJ background as described previously (33). *pRb* $^{+/-}$ mice were maintained on C57BL/6 ×129/Sv mixed background. Genotyping was done on tail DNA by PCR.

Cytokine array analysis. Biotin label-based mouse antibody arrays were obtained from Raybiotech (Norcross, GA). The hybridization was performed following the instructions from the user manual. The intensities of signals were quantified using AlphaImager software.

Effect of cyclin D1 knockout medium on the differentiation of CD34+ hematopoietic stem cells into bone marrow-derived dendritic cells. The effect of cyclin D1 knockout medium was tested on the differentiation of CD34$^{+}$ hematopoietic stem cells (HSCs) into bone-marrow derived dendritic cells (BMDCs). Briefly, 30 x $10^6$ BM cells were obtained from 6- to 10-week-old C57BL/6J mice (purchased from The Jackson Laboratory, Bar Harbor, ME) cultured in medium obtained from wild type or Rb KO, Rb/D1 KO or D1 KO animals and supplemented with 20% of Flt3L (Fms-related tyrosine kinase 3 ligand) containing supernatant, produced from an SP2/0 transfected cell line that secretes murine recombinant Flt3L These cells were kindly provided by Dr. Robert Rottapel, Ontario Cancer Institute, Toronto Medical Discovery Tower, Toronto, Canada. The cells were maintained undisturbed at 37°C in 5% $CO_2$ and 90% relative humidity. On day 9, the cells were collected by gentle trypsinization and phenotyped for stem cell marker (CD34) as well as myeloid (CD11c+/CD11b+/B220-) and plasmacytoid (CD11c+/CD11b-/B220+) DC markers using specific antibodies for flow cytometry.




G2, G3 and G4 *vs* G1

| | Coef | Hazard | SE | Z | PValue |
|---|---|---|---|---|---|
| G2 | 0.048012 | 1.049183 | 0.387362 | 0.123946 | 0.901358 |
| G3 | 0.628365 | 1.874544 | 0.359643 | 1.747194 | 0.080604 |
| G4 | 0.72775 | 2.070417 | 0.314714 | 2.31242 | 0.020755 |

Figure 1. Human breast cancer miRNA hierarchical clustering define 4 distinct groups. Expression of miRNA across samples compiled from four datasets. Rows and columns are sorted using hierarchical clustering with Pearson correlation and average linkage. Hierarchical clustering is used to classify samples according to miRNA expression profiles. The color of the sample dendrogram indicates which samples are classified into miRNA expression clusters (G1-G4). Green represents down-regulation, red represents up-regulation. Groups identified by mRNA molecular subtypes (red = basal, blue = luminal A, purple = luminal B, orange = HER2+, green = normal-like). (B) Kaplan-Meier plot of relapse-free survival in breast cancer for miRNA subtypes. Groups identified by hierarchical clustering of miRNA expression profiles (Figure 1). Log-rank p-value = 0.04, hazard ratio = 1.05. (B) Kaplan-Meier plot of relapse-free survival in breast cancer for molecular subtypes. Log-rank p-value = 1.6E-4, hazard ratio = 1.87. (C). Table of hazard ratio in which miRNA subtype is compared for outcome comat (from Yu, Z., Gormley, M., Chen, H., Ju, X., Wang, M., Li, Z., Ertel, A., Fortina, P., Tozeren, A., Pestell, RG. Cyclin D1-Mediated MicroRNA Expression Signature In Breast Cancer Subtype And Outcome. In preparation)

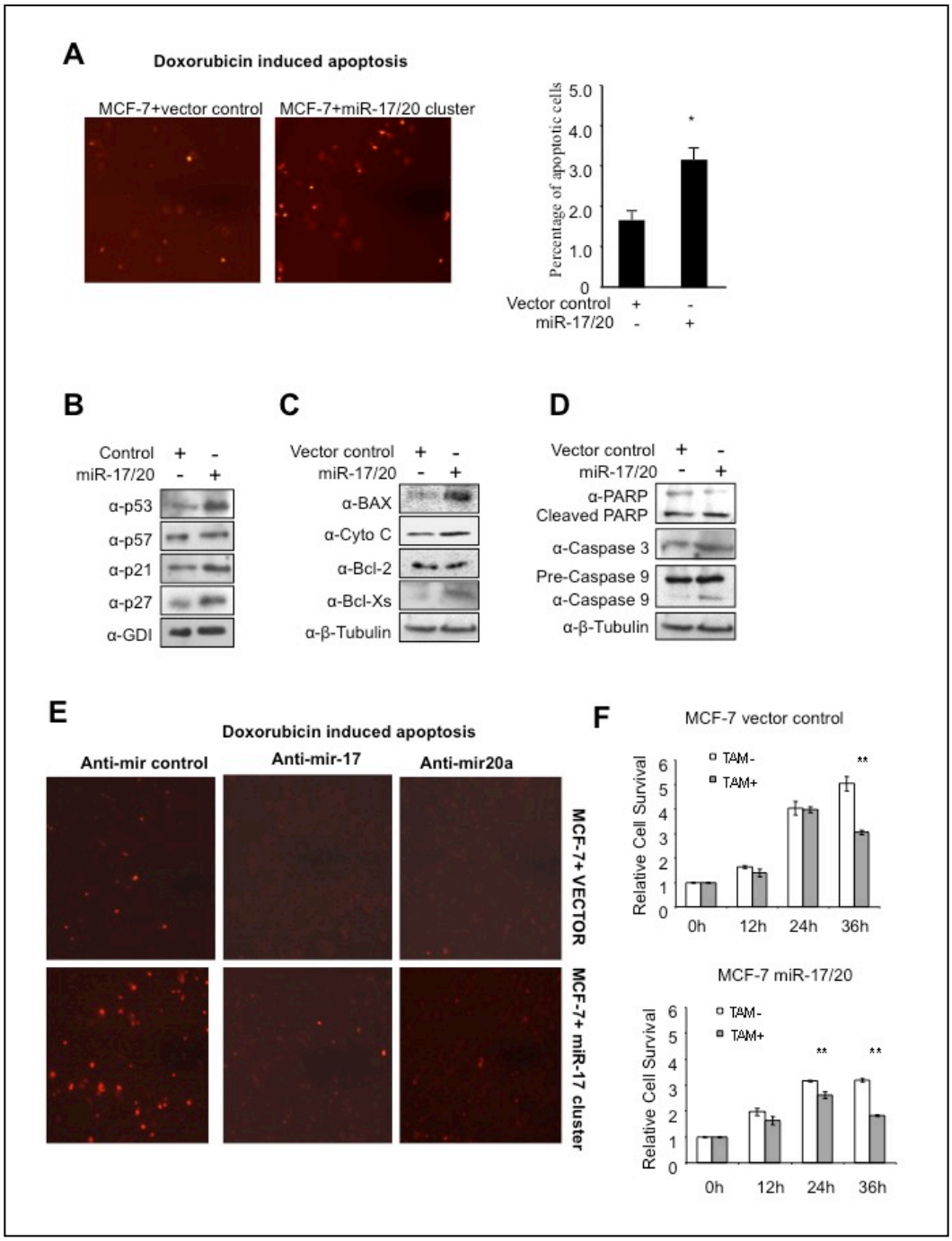



Figure 2. mir17/20 sensitizes human breast cancer cells to tamoxifen induced cell death. Human MCF7 breast cancer cells were transduced with the mir17-20 expression vector and cell death in response to DNA damage inducing agents assessed using annexin V staining for apoptosis (quantitated as mean ± SEM for N>5 separate experiments. (B-D) Western blot of apoptotic signaling pathways with antibodies as indicated in the figure showing the induction of p53, BAX, Bcl-Xs and enhanced caspase cleavage. (E)

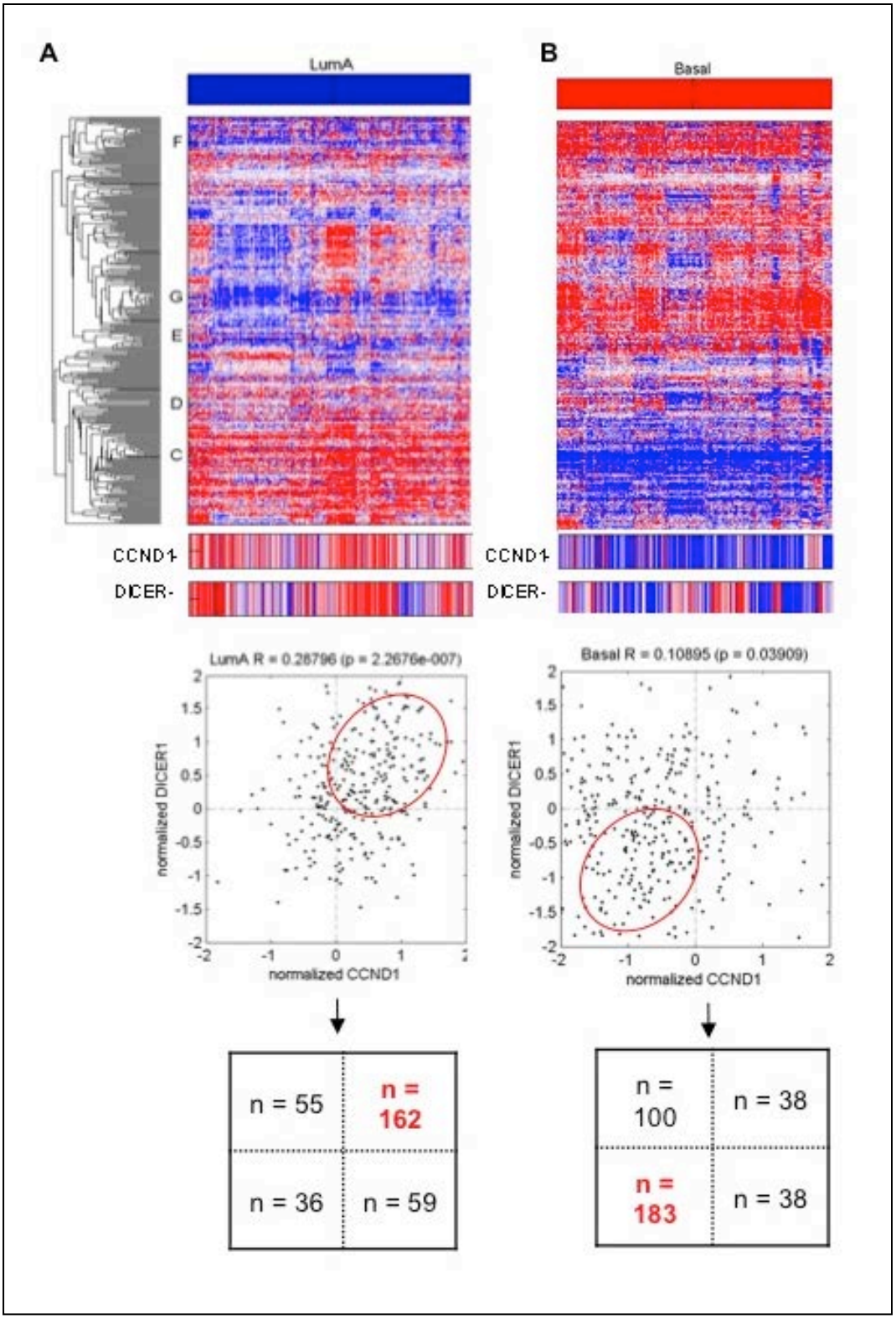



Figure 3. A positive correlation analysis between Dicer and cyclin D1a expression in breast cancer patients. (A) Tree view display of gene expression from breast cancer patient samples for luminal A and basal genetic subtype. 2D-scatter plot for the elevated Dicer expression and high expression of cyclin D1a in luminal A breast cancer subtype (N=312). (B) 2D-scatter plot for the decreased Dicer expression and low expression of cyclin D1a in basal breast cancer subtype (N=359). The number of breast cancer patient samples found in each of the four quadrants is indicated. Plots are labeled with Pearson's correlation coefficient R and the correlation p-value. FROM: Yu,Z., Wang, L. Ju, X., Wang, M., Ertel, A., Loro E., Li,Z., Wu, K., Gormley, M., Wang , C., Fortina, P., Tozeren, A., and Pestell, RG. Cyclin D1 Induction of Dicer Governs MicroRNA processing and Expression in Breast Cancer EMBO J In review

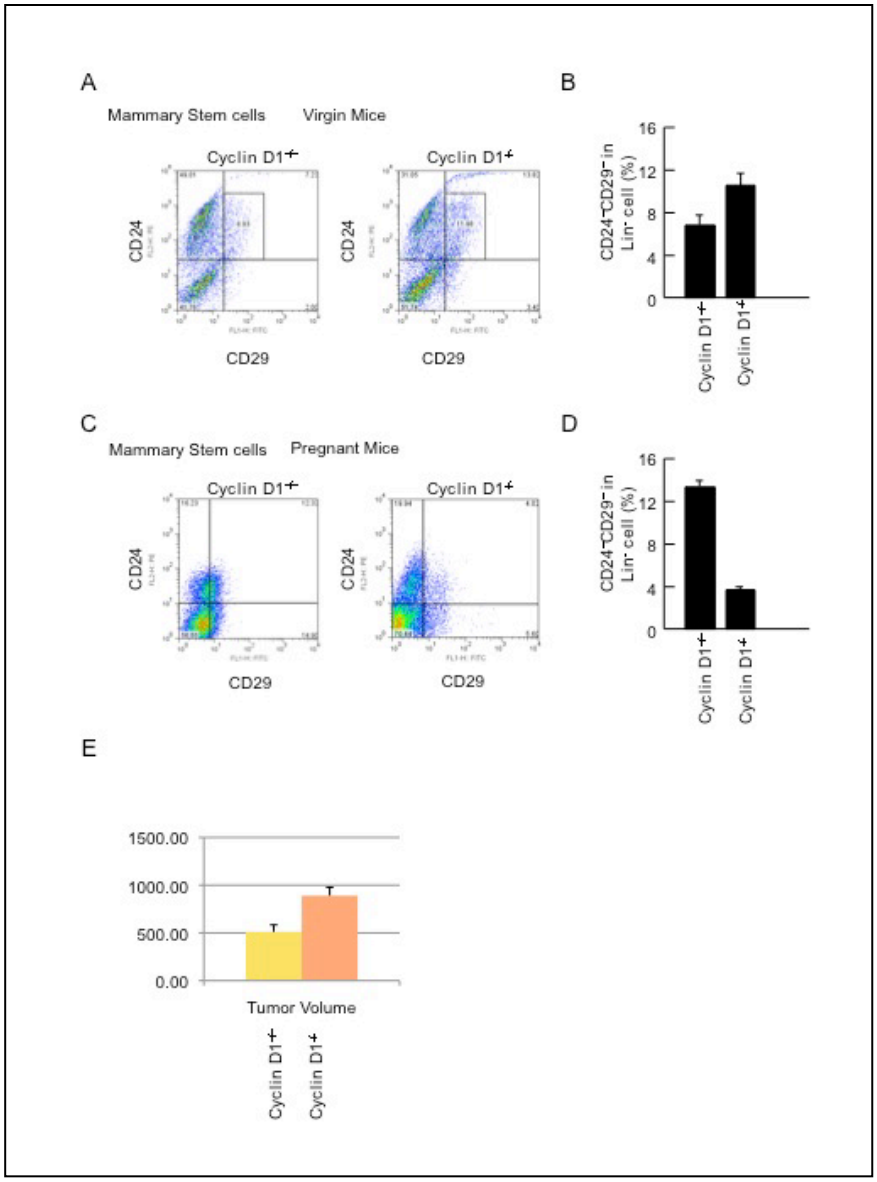



Figure 4. Cyclin D1 expands mammary stem cell population. FACS based cell sorting for mammary epithelial stem cells using the CD24/CD29-lin- cells in either virgin (wt vs *cyclin D1*$^{-/-}$,) mice or (C,D) pregnant mice mammary glands.

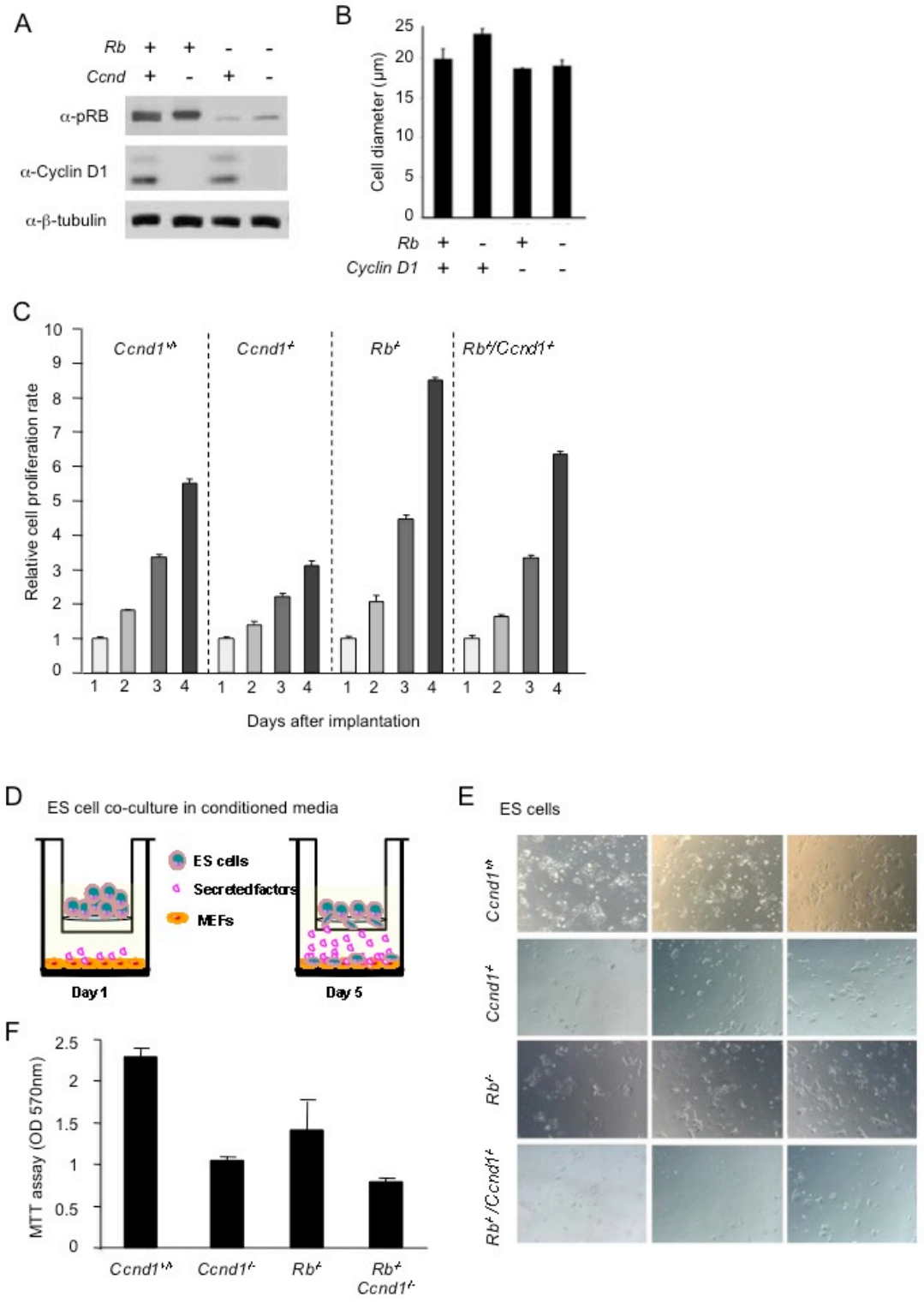



**Figure 5**. **Cyclin D1 induces stem cell expansion via heterotypic signals.** A). analysis of MEFs derived from cyclin D1-/- Rb-/- and cyclin D1 Rb-/- mice with subsequent analysis of cell diameter and ©. Cell proliferation. (D) Schematic representation of co-culture in which MEFs are coincubated with murine embryonic stem cells, and their growth assessed by (E) phase contrast and (F) MTT activity for cell proliferation.

Presentations

2009

Jun 10-15 "A cyclin D1/miRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation." Yu Z, Wang C, Wang M, Li Z, Casimiro MC, Liu M, Wu K, Whittle J, Ju X, Hyslop T, McCue P, Pestell RG. Keystone Symposia on Molecular and Cellular Biology, Keystone, CO.

Nov 25-27 "Invasion and Metastasis in Breast Cancer-New Mechanism and Role of miRNA." Pestell RG. Catholic University, Rome, Italy.

Nov 30 "Invasion and Metastasis in Breast Cancer-New Mechanism and Role of miRNA." Pestell RG. Sapienza University of Rome, Rome, Italy.

Dec 11 "Invasion and metastasis – new mechanisms and role of miRNA." Pestell RG. Queen Elizabeth II Medical Center, Perth, Australia.

2010

Jun 2 "Invasion and metastasis – new mechanisms and role of miRNA". Pestell RG. Center for Cancer Research at Massachusetts General Hospital, Boston, MA.

Dec 9 "Invasion and metastasis – new mechanisms and role of miRNA." Pestell RG. Lankenau Institute for Medical Research (LIMR).

Dec 16 "Invasion and metastasis – new mechanisms and role of miRNA." Pestell RG. University of Western Australia, Raine Foundation Lecture.

2011

Mar 28-30 "microRNA 17/20 mediates cellular invasion and tumor metastasis in breast cancer by heterotypic signaling." Yu Z, Willmarth NE, Zhou J, Katiyar S, Wang M, Liu Y, McCue PA, Quong AA, Lisanti MP, Pestell RG. Seventh Annual microRNA in Human Disease and Development Conference, Cambridge, MA.

Publications

1. Yu Z, Pestell RG. "MicroRNA and Cancer Stem Cells." Chapter in: MicroRNAs in Cancer Translational Research. Springer. (2011) p. 373 - 388.

2. 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. Epub 2010 Apr 20.

3. Yu Z, Baserga R, Chen L, Wang C, Lisanti MP, Pestell RG. microRNA, cell cycle, and human breast cancer. Am J Pathol. 2010 Mar; 176(3): p. 1058-64.

4. Yu Z, Pestell RG. Small Non-coding RNAs Govern Mammary Gland Tumorigenesis. J Mammary Gland Biol Neoplasia. 2012 Mar;17(1):59-64. Epub 2012 Mar 1.

5. Yu Z, Tozeren A, Pestell RG. "Function of miRNAs in Tumor Cell Proliferation." MicroRNA in Cancer. Editor: Dr. Suresh Alahari. Springer Science+Business Media. Dordrecht. 2013.

6. Yu Z, Li Y, Fan H, Liu Z, Pestell RG. miRNAs Regulate Stem Cell Self-Renewal and Differentiation. Front. Gene. 3:191. doi: 10.3389/fgene.2012.00191.

7. Yu Z, Wang L, Ju X, Wang M, Ertel A, Loro E, Li Z, Wu K, Gormley M, Wang C, Fortina P, Tozeren A, Pestell RG. Cyclin D1 Induction of Dicer Governs MicroRNA processing and Expression in Breast Cancer. Nature Communications. In review.

8. Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. Cyclin D1-Mediated MicroRNA Expression Signature In Breast Cancer Subtype And Outcome. In preparation.

9. Wu K, Yu Z, Li Z, Crosariol M, Prisco M, Rui H, Casimiro MC, Lisanti MP, Pestell RG. Cyclin D1-dependent microRNA signaling pathways enhances stem cell expansion via heterotypic signaling. In preparation.

10. Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. mir17-20 sensitizes breast cancer cells to tamoxifen mediated apoptosis, via AKT1 In preparation.

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

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

_____Yes
__X__No

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

_____Yes
__X__No

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

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

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

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

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

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

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

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

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

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

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

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

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

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

**20. Articles Submitted to Peer-Reviewed Publications**.

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

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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

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

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. microRNA 17/20 inhibits cellular invasion and tumor metastasis in breast cancer by heterotypic signaling. | Yu Z, Willmarth NE, Zhou J, Katiyar S, Wang M, Liu Y, McCue PA, Quong AA, Lisanti MP, Pestell RG. | Proceedings of the National Academy of Science | Feb 2010 | ☐Submitted<br>☐Accepted<br>☒Published |
| 2. microRNA, cell cycle, and human breast cancer. | Yu Z, Baserga R, Chen L, Wang C, Lisanti MP, Pestell RG. | American Journal of Pathology | Oct 2009 | ☐Submitted<br>☐Accepted<br>☒Published |
| 3. Small Non-coding RNAs Govern Mammary Gland Tumorigenesis. | Yu Z, Pestell RG. | Journal of Mammary Gland Biology and Neoplasia | Jan 2012 | ☐Submitted<br>☐Accepted<br>☒Published |
| 4. miRNAs Regulate Stem Cell Self-Renewal and Differentiation. | Yu Z, Li Y, Fan H, Liu Z, Pestell RG. | Frontiers in Genetics | June 2012 | ☐Submitted<br>☒Accepted<br>☐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 submit the following articles currently in preparation:

Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. *Cyclin D1-Mediated MicroRNA Expression Signature In Breast Cancer Subtype And Outcome*. In preparation.

Wu K, Yu Z, Li Z, Crosariol M, Prisco M, Rui H, Casimiro MC, Lisanti MP, Pestell RG. *Cyclin D1-dependent microRNA signaling pathways enhances stem cell expansion via heterotypic signaling*. In preparation.

Yu Z, Gormley M, Chen H, Ju X, Wang M, Li Z, Ertel A, Fortina P, Tozeren A, Pestell RG. *mir17-20 sensitizes breast cancer cells to tamoxifen mediated apoptosis, via AKT1*. 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.

A new breast cancer prognostic was developed based on miRNA of >400 patients with breast cancer.

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

1) New breast cancer prognostic developed using miRNA of >400 patients
2) Evidence of a secreted factor that promotes stem cell expansion may be of therapeutic value

**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__X_____ No_____

If yes, please describe your plans:

miRNA gene clustering for outcome

**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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

## BIOGRAPHICAL SKETCH

| NAME<br>**PESTELL, RICHARD G., M.B., B.S., M.D., PH.D.** | POSITION TITLE<br>**Professor and Chairman** |
|---|---|
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>**RPESTELL** | |

| INSTITUTION AND LOCATION | DEGREE<br>*(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 | Post Doc | 1993 | Molecular Biology |
| New York University, New York, NY | MBA | 2011 | |

**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 the retinal determination gene network (DACH1/EYA) 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. We demonstrated that acetylation governs functions including tumor growth and have identified DACH1 as an acetylated protein binding to p53. We are determining mechanisms by which this DACH1-mediated cell-fate determination pathway controls tumor growth. We have contributed to a new model of cancer via stem cell expansion. I have successfully administered R01 and P30 Projects (e.g. staffing, research protections, budget); collaborated with other researchers and produced ~425 peer-reviewed publications. I have mentored students for >18 years, many of which have obtained academic positions all over the world.

**B. Positions and Honors**

| | |
|---|---|
| 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 more than 425 publications)

1. Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID, and Rosen EM. *BRCA1 inhibition of estrogen receptor signaling in transfected cells.* **Science**. 1999 May 21; 284(5418): p. 1354-6.
2. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, and Darnell JE Jr. *Stat3 as an oncogene.* **Cell**. 1999 Aug 6; 98(3): p. 295-303.
3. Wu K, Yang Y, Wang C, Davoli MA, D'Amico M, Li A, Cveklova K, Kozmik Z, Lisanti MP, Russell RG, Cvekl A, Pestell RG. *DACH1 inhibits transforming growth factor-beta signaling through binding Smad4.*
   **J Biol Chem**. 2003 Dec 19;278(51):51673-84. Epub 2003 Oct 2.
4. Yang Y, Stopka T, Golestaneh N, Wang Y, Wu K, Li A, Chauhan BK, Gao CY, Cveklová K, Duncan MK, Pestell RG, Chepelinsky AB, Skoultchi AI, Cvekl A. *Regulation of alphaA-crystallin via Pax6, c-Maf, CREB and a broad domain of lens-specific chromatin*. **EMBO J**. 2006 May 17;25(10):2107-18. Epub 2006 May 4.
5. Sunde JS, Donninger H, Wu K, Johnson ME, Pestell RG, Rose GS, Mok SC, Brady J, Bonome T, Birrer MJ. *Expression profiling identifies altered expression of genes that contribute to the inhibition of transforming growth factor-beta signaling in ovarian cancer*. **Cancer Res**. 2006 Sep 1;66(17):8404-12.
6. Wu K, Li A, Rao M, Liu M, Dailey V, Yang Y, Di Vizio D, Wang C, Lisanti MP, Sauter G, Russell RG, Cvekl A, Pestell RG. *DACH1 is a cell fate determination factor that inhibits cyclin D1 and breast tumor growth*. **Mol Cell Biol**. 2006 Oct;26(19):7116-29.
7. Wu K, Liu M, Li A, Donninger H, Rao M, Jiao X, Lisanti MP, Cvekl A, Birrer M, Pestell RG. *Cell fate determination factor DACH1 inhibits c-Jun-induced contact-independent growth*. **Mol Biol Cell**. 2007 Mar;18(3):755-67. Epub 2006 Dec 20.
8. Wu K, Katiyar S, Li A, Liu M, Ju X, Popov VM, Jiao X, Lisanti MP, Casola A, Pestell RG. *Dachshund inhibits oncogene-induced breast cancer cellular migration and invasion through suppression of interleukin-8*. **Proc Natl Acad Sci U S A**. 2008 May 13;105(19):6924-9. Epub 2008 May 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):3347-55. Epub 2009 Apr 7.
10. Popov VM, Zhou J, Shirley LA, Quong J, Yeow WS, Wright JA, Wu K, Rui H, Vadlamudi RK, Jiang J, Kumar R, Wang C, Pestell RG. *The cell fate determination factor DACH1 is expressed in estrogen receptor-alpha-positive breast cancer and represses estrogen receptor-alpha signaling*. **Cancer Res**. 2009 Jul 15;69(14):5752-60.
11. Popov VM, Wu K, Zhou J, Powell MJ, Mardon G, Wang C, Pestell RG. *The Dachshund gene in development and hormone-responsive tumorigenesis*. **Trends Endocrinol Metab**. 2010 Jan;21(1):41-9. Epub 2009 Nov 5. Review.