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:** The Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2013 – 06/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** 4100062226

6. **Project Number and Title of Research Project:** 3: Molecular Basis of BRCA1 and PALB2 Tumor Suppression

7. **Start and End Date of Research Project:** 01/01/2013 – 06/30/2014

8. **Name of Principal Investigator for the Research Project:** Ramin Shiekhattar, 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:

   $281,647.64

   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
Shiekhattar	Principal Investigator	42%	$129,842
Cesaroni	Postdoctoral Fellow	100%	$ 23,426
Cooch	Research Assistant	19%	$ 9,966
Gardini	Postdoctoral Fellow	29%	$ 20,278

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

Last Name, First Name	Position Title	% of Effort on Project
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___X_____ No_____

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

PA Breast Cancer Coalition: $50,000

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_____ No____x_____

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:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

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

Yes____x_____ No_____

If yes, please describe your plans:

We are planning to submit an RO1 grant.

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

Recent work in murine models has suggested that the BRCA1 C Terminus (BRCT) domain of BRCA1 gene is the required module for its tumor suppressor activity (Shakya et al., Science, 2011). Therefore, it would be important to uncover whether the BRCT is critical for NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) or retinoic acid (RA)-signaling and understand the molecular basis for such requirement. We plan to identify the functional domains in the tumor suppressor genes BRCA1 and PALB2 that are required for transcriptional activation and to dissect the molecular basis for the requirement of such domains. We will explore the Ring Finger and BRCT domains in BRCA1 and the chromatin interaction domain in PALB2.

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_____

The Wistar Institute Genomic Facility (the "Facility") performed an extensive amount of work on this project. There has been extensive collaboration between the Facility and our research group, including technical troubleshooting and the creation of improved pipelines of bioinformatics analysis.

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.

Project rationale

We postulate that breast and ovarian cancer susceptibility genes (BRCA1 and PALB2) are transducers of multiple signaling pathways and their functional inactivation in breast and ovarian epithelial cells lead to a loss of responsiveness to extracellular signals. We envision that the breast and ovarian cancer susceptibility genes function in transcriptional regulation to mediate mammary epithelial cell morphogenesis and differentiation. Our working hypothesis is that the tumor suppressor activity of BRCA1 and PALB2 is due to their function as co-activators of transcription for growth inhibitory signals.

Specific Aims

1. Determine the molecular basis by which BRCA1 and PALB2 activate transcription in response to NF-kB and RA.
2. Determine how cancer-causing mutations in BRCA1 alter their function at their target genes.

Summary of research results

Association of BRCA1 and PALB2 with elongating RNAPII

The chromatin residence of BRCA1 and PALB2, occupying the 5'-end and extending into the body of the transcriptionally active genes, suggested a functional association between BRCA1/PALB2 and the elongating form of RNAPII. Indeed, recent *in vitro* studies described the association of BRCA1 with the C-terminal domain (CTD) of Ribonucleic Acid Polymerase II (RNAPII), and the ubiquitination of elongating polymerase by the BRCA1/BARD1 heterodimer. To explore the association of BRCA1 and PALB2 with RNAPII *in vivo*, we compared our genome-wide occupancy of BRCA1 and PALB2 to an RNAPII Chromatin Immunoprecipitation Sequencing (ChIP-seq) experiment that we previously performed in MCF10A cells using polyclonal antibodies (N-20), which recognize RPB1 independent of its phosphorylation status. Analysis of 373 highly active genes revealed a similar pattern of occupancy for RNAPII and the breast cancer susceptibility proteins (Figure 1A).

To directly analyze the elongating form of RNAPII, we performed ChIP-seq using antibodies against the Ser2-phosphorylated form of RNAPII (P-Ser2), which corresponds to the elongating form of RNAPII. Genes targeted by BRCA1 and PALB2 display high levels of Ser2 phosphorylation (Figure 1B). Importantly, the average profile of the elongating form of RNAPII is highly similar to that of PALB2 at highly active genes (Figure 1C), which is accumulating at the 3' end of genes. Indeed, unbiased clustering of genes occupied by PALB2 and elongating form of RNAPII revealed a strikingly similar profile at all Reference Sequence (RefSeq) genes (Figure 1C). To assess whether the association of BRCA1 and PALB2 with active genes requires the elongating form of RNAPII, we inhibited transcriptional elongation using Flavopiridol, an inhibitor of RNAPII elongation. Treatment of MCF10A cells with Flavopiridol

resulted in a concomitant decrease in transcriptional activity, as well as the occupancy of BRCA1, PALB2 and elongating form of RNAPII at the 3'-end of all genes tested (Figure 1D). Taken together, our results point to an intimate functional association of BRCA1 and PALB2 with the elongating form of RNAPII.

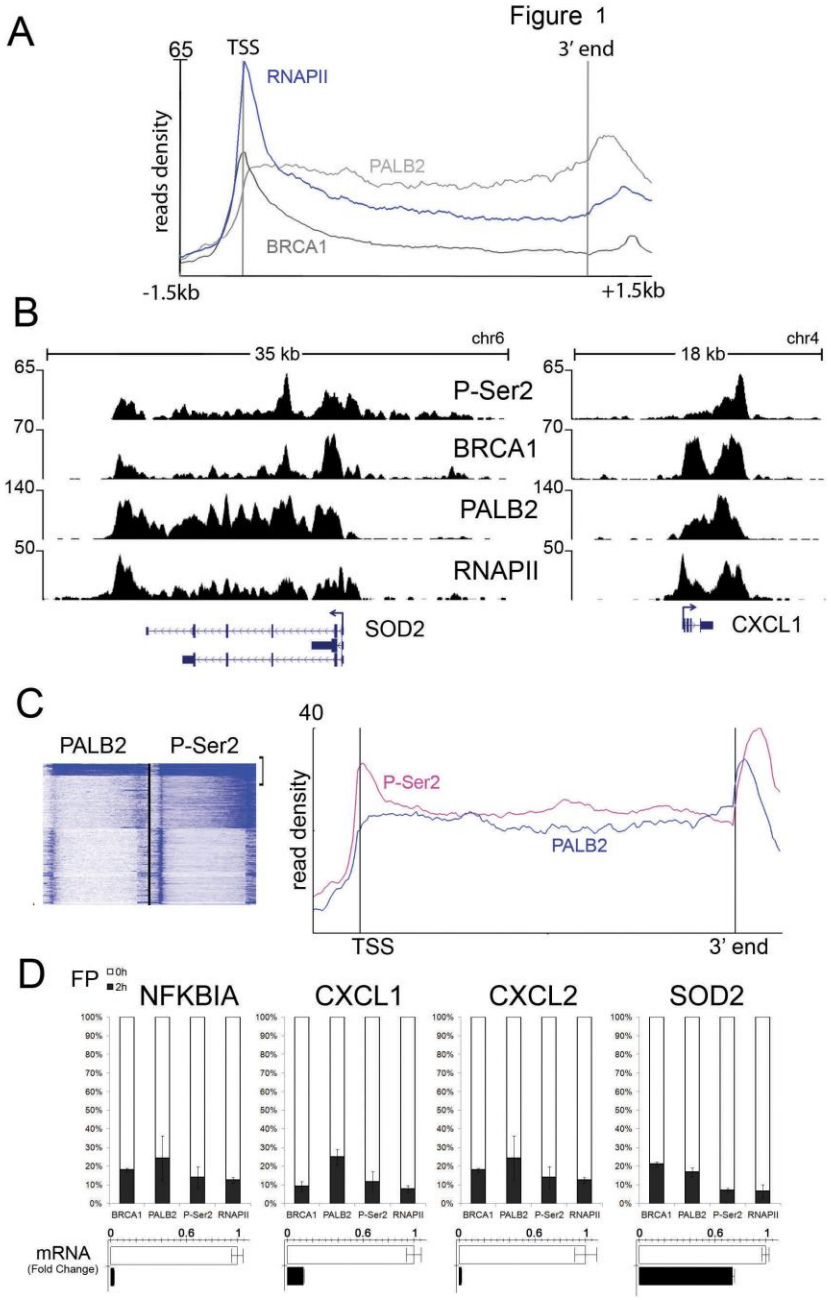


Figure 1. The breast cancer genes mirror RNA Polymerase II occupancy and are functionally linked with its elongating form. (**A**) The average profile of RNAPII, BRCA1 and PALB2 read density of class I genes (n=373) in MCF10A cells. The average read densities of RNAPII peak at the transcription start site and remains elevated across entire gene body similar to the BRCA proteins. (**B**) Genome-wide analysis of elongating RNAPII was performed in MCF10A cells using antibodies against phosphorylated Ser2 of the CTD (P-Ser2). Snapshots of the aligned-reads for P-Ser2, PALB2, BRCA1 and RNAPII show a compelling overlap between the four ChIP-seq datasets at two loci representative of the target genes of the BRCA1/PALB2 complex. (**C**) Unbiased clustering of PALB2 and P-Ser2 reveals an intimate association between the most recently discovered gene implicated in familial breast cancer and

the elongating form of RNA Polymerase II on the left panel. The right panel depicts the average read profiles of the most actively transcribed genes (comprising of approximately 300 genes). (**D**) Inhibition of elongation abrogates BRCA1 and PALB2 recruitment at target genes. MCF10A cells were treated with Flavopiridol for 2h and subjected to ChIP analysis at the 3'-end of a group of highly active genes. The stacked bars indicate the residual amount of BRCA1, PALB2, RNAPII and P-Ser2 after flavopiridol treatment, relative to untreated cells (100%). Average of three IPs are shown. A dramatic decrease in elongating RNAPII is mirrored by loss of over 70-80% of the BRCA1-PALB2 complex at chromatin. RT-PCR analysis of the mRNA levels after Flavopiridol treatment is also shown.

The role of BRCA1 and PALB2 in responsiveness to Tumor Necrosis Factor-alpha (TNF-alpha) stimulation

We examined the role of BRCA1 and PALB2 in TNF-alpha responsiveness in the breast cancer cell line MCF7. Treatment of MCF7 cells with 10 ng/ml TNF-alpha for one hour resulted in increased occupancy of p65/RelA, BRCA1 and PALB2 at the promoter of candidate NF-kB target genes (Figure 2A). Moreover, in contrast to the requirement for p65/RelA in the recruitment of BRCA1 and PALB2, depletion of BRCA1 or PALB2 did not affect the occupancy of p65/RelA (Figure 2B, C). We next performed gene expression analysis using microarrays to determine the genome-wide responsiveness of MCF7 cells following treatment with TNF-alpha. Overall, 33 genes displayed a significant activation following treatment of MCF7 cells with TNF-alpha (log2(foldChange)>0.4, $p<0.01$). We validated the response to TNF-alpha using real-time PCR for eight genes in three independent experiments. Additionally, we examined *CXCL1*, *CXCL3*, *NFKBIA*, and *SOD2* (which, while responsive to TNF-alpha using real-time polymerase chain reaction (PCR), were not reliably detected on the microarray). While depletion of p65/RelA resulted in the loss of TNF-alpha responsiveness in 31 of 33 genes represented on the array and all four additional genes examined (35 of 37 genes examined), BRCA1 depletion led to a diminished responsiveness to TNF-alpha for a subset of genes (18 of 37; Figure 2D). A smaller group of genes (10 of 37) displayed a significant decrease in their response following PALB2 depletion (Figure 2E). Additionally, a large number of TNF-alpha responsive genes (24 of 37) showed a significant decrease in their basal activity following depletion of p65/RelA, BRCA1 or PALB2 (Figure 2D, 2E, $p<0.05$). It is important to note that infection of MCF10A or MCF7 cells with viral vectors used in our depletion studies may activate a component of the NF-kB pathway, which may contribute to the basal NF-kB responsiveness in MCF10A and MCF7 cells. Taken together, depletion of p65/RelA had a greater effect on TNF-alpha responsiveness than that of BRCA1 or PALB2 depletion, reflecting the fact that there may be other NF-kB co-activators and that BRCA1/PALB2 may only confer a component of the TNF-alpha response.

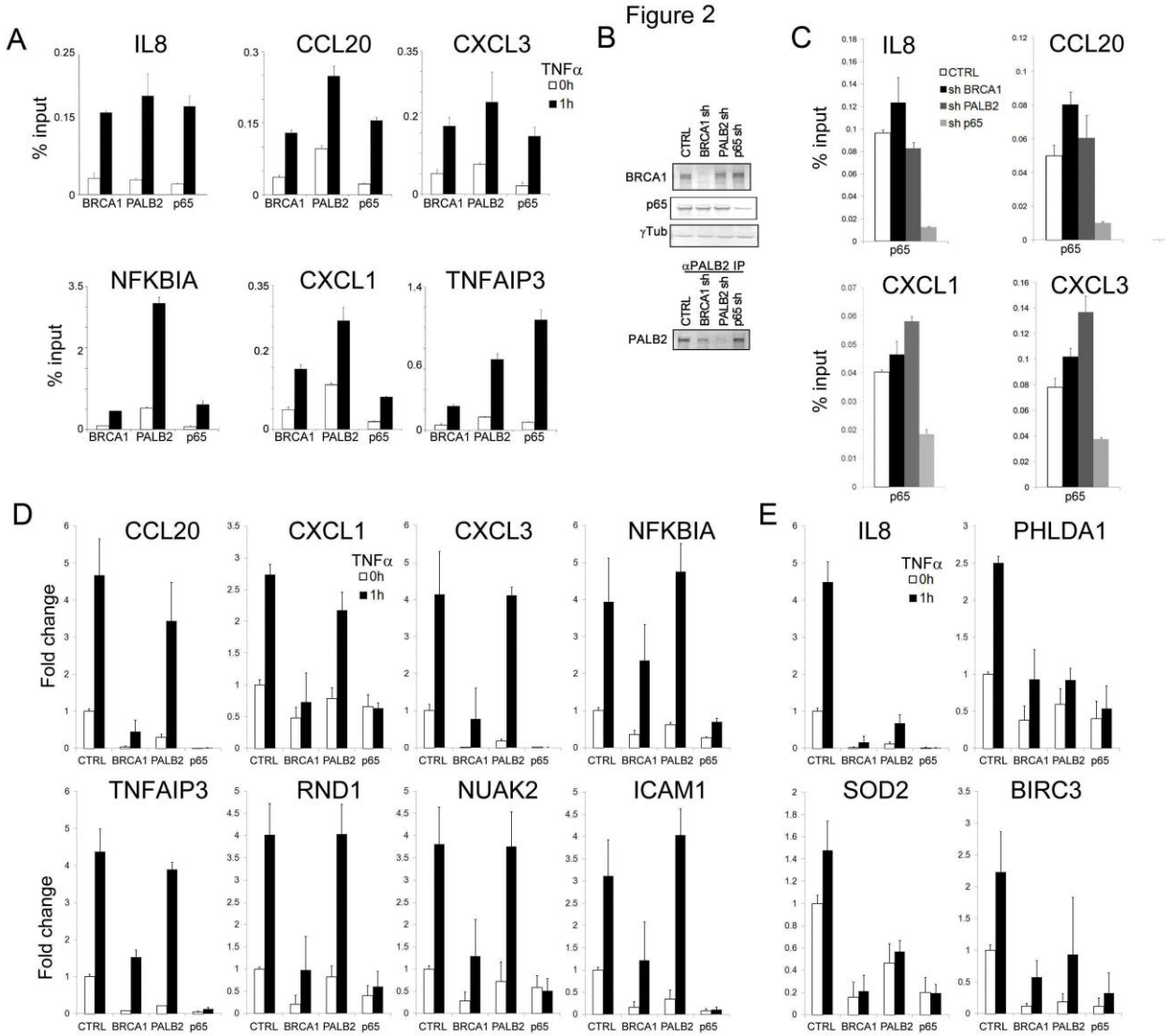


Figure 2. BRCA1 and PALB2 affect TNF-alpha-mediated response at NF-kB target genes. (**A**) ChIP analysis of TNF-alpha stimulated MCF7 cells shows a substantial recruitment of p65/RelA, BRCA1 and PALB2 at a set of target genes. Cells were analyzed after 1h of treatment. (**B**) Depletion of BRCA1, PALB2 and p65 in MCF7 cells with lentiviral transduced shRNAs. Due to PALB2 antibodies poorly performing in a total extract, we immunoprecipitated PALB2 before testing its depletion by immunoblot. (**C**) Impact of BRCA1, PALB2 and p65/RelA depletion on p65/RelA association upon TNF-alpha stimulation in MCF7 cells. ChIP analysis was performed after 1h stimulation with TNF-alpha and reveals that neither BRCA1 nor PALB2 depletion affects p65/RelA binding to the TSS of its target genes. (**D**) Transcriptional effects of TNF-alpha at selected genes in MCF7 cells. Depletion of BRCA1 but not PALB2 significantly impairs activation in all genes tested ($p<0.05$) with the exception of *NFKBIA* ($p<0.02$). Most genes display attenuation of basal transcription following BRCA1, PALB2 and p65 depletion. (**E**) Transcriptional effects of TNF-alpha at selected genes in MCF7 cells. Depletion of BRCA1 and PALB2 impairs gene activation mediated by NF-alpha at *IL8*, *PHLDA1*, *BIRC3* (as resulted from the microarray analysis) and *SOD2* (uncovered as a target gene in MCF10A cells). *SOD2*, *IL8* and *BIRC3* also show a pronounced attenuation of basal transcription. All four genes displayed a significant attenuation of their responsiveness following depletion of BRCA1 and PALB2 in three independent experiments ($p<0.05$).

Determine how cancer-causing mutations in BRCA1 alter their function at their target genes.
We have started to investigate whether the transcriptional activity of BRCA1 and PALB2 is relevant to cancer. We found that BRCA1 mutant cell line HCC1937 displayed aberrant responsiveness to TNF-alpha. HCC1937 cells synthesize a truncated BRCA1 protein that is a product of a disease-producing mutant allele (5382insC) and no wild type protein (Chen et al, 1998; Tomlinson et al, 1998). We used HCC1937 cells that were reconstituted with either wild type BRCA1 construct or vector alone (Scully et al, 1999). We observed a robust enhancement of HCC1937 cells reconstituted with wild type BRCA1 compared to the parental lines expressing an empty vector, consistent with the role for BRCA1 in TNF-alpha (Figure 3A, also see previous annual report). To gain further insight into the mechanism by which BRCA1 and PALB2 mediate responsiveness to TNF-alpha we asked whether depletion of BRCA1 or PALB2 decrease the recruitment of total RNAPII, its serine 2 or serine 5 phosphorylated forms. Recruitment of RNAPII and its phosphorylated forms is substantially diminished following BRCA1 or PALB2 depletion in genes that displayed corresponding changes in their transcription (Figure 3B).

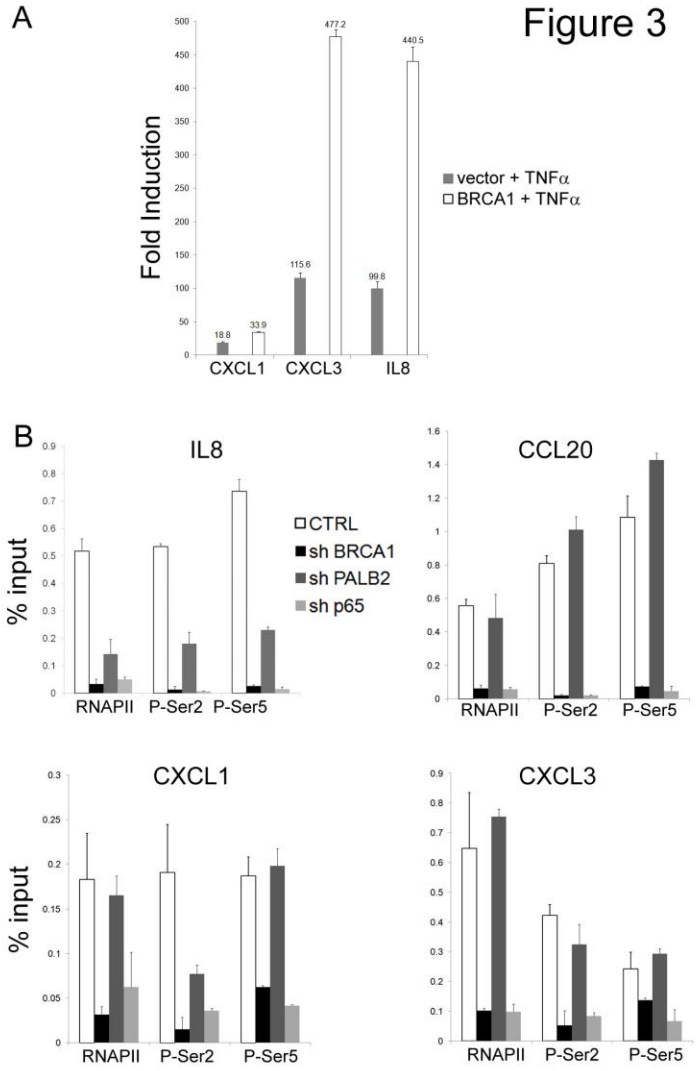


Figure 3. Requirement of BRCA1 for TNF-alpha activation
(A) Response to TNF-alpha was investigated in HCC1937 cells, which carry one defective allele of BRCA1. Cells stably transfected with the empty vector or stably reconstituted with wild type BRCA1 were stimulated with TNF-alpha for 1h. Fold induction relative to t=0h for each cell line was calculated. Data are normalized to beta-glucuronidase (GUSB) expression.
(B) Analysis of RNAPII and its phosphorylation status in MCF7 cells depleted of BRCA1, PALB2 or p65. qChIP was performed after TNF-alpha stimulation and reveals a substantial impairment of RNAPII recruitment after PALB2 and BRCA1 depletion. BRCA1 also impairs CXCL1, CXCL3 and CCL20 activation (Figure 2D), which reflects in a severe reduction of RNAPII recruitment.

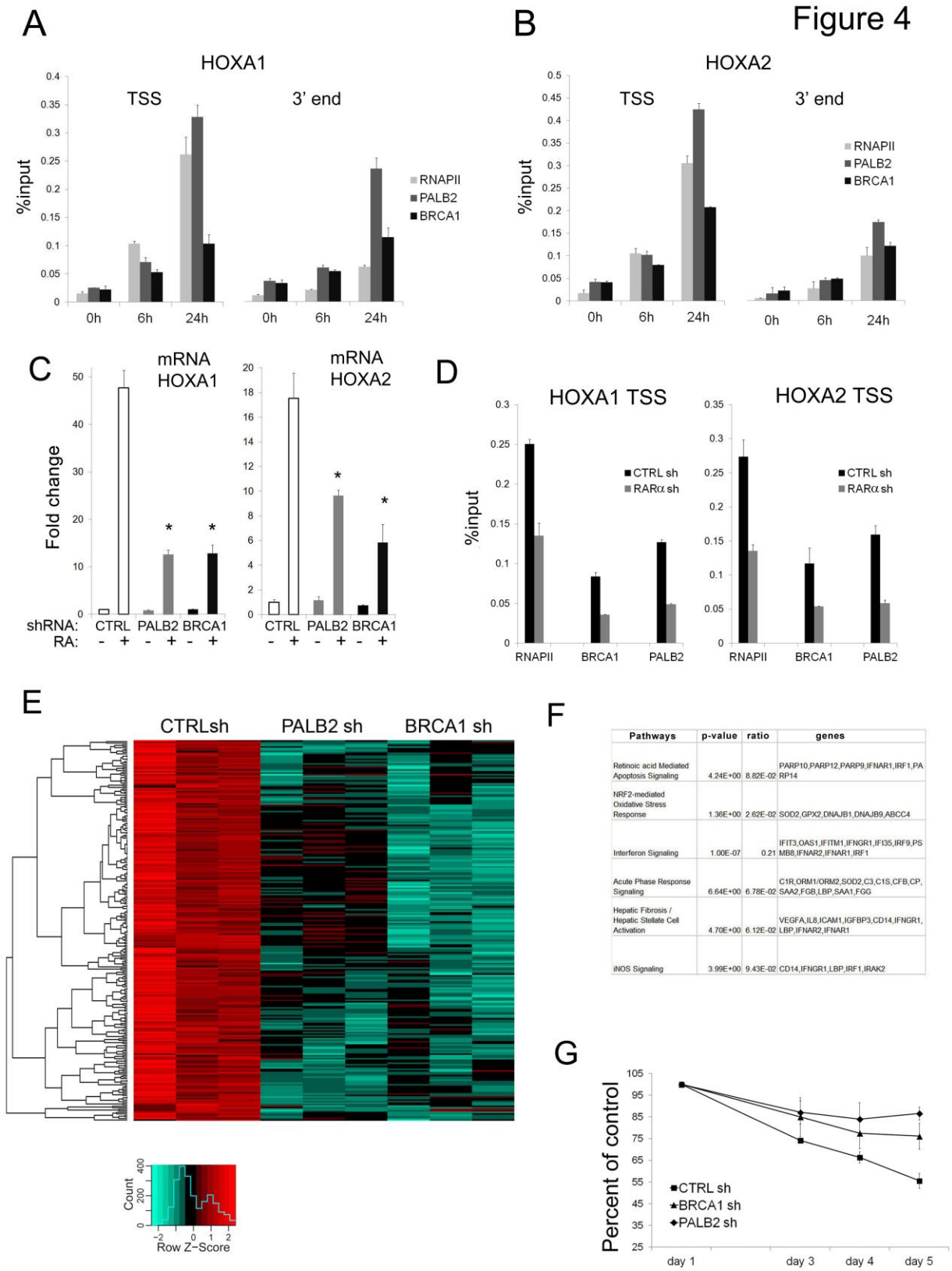
Role of BRCA1 and PALB2 in Retinoic Acid signaling

We then turned our attention to the retinoic acid (RA) signaling, which was previously shown to have anti-proliferative effects in breast cancer cells (Donato & Noy, 2005; Hua et al, 2009). Strikingly, the RA signaling showed a compelling requirement for BRCA1 and PALB2. Treatment of MCF7 cells with RA led to a concomitant increase in the recruitment of RNAPII, BRCA1 and PALB2 on HOXA1 and HOXA2 genes supporting a direct function for these breast cancer susceptibility proteins in RA responsiveness (Figure 4A, B). Importantly, BRCA1 and PALB2 are not only recruited at the TSS following stimulation with RA, but also accumulate at the 3' end of genes (Figure 4A, B). We next turned to examining the functional impact of BRCA1 and PALB2 on RA responsiveness. While treatment with RA induced a ~50 and ~20 fold activation of HOXA1 and HOXA2 genes, respectively, depletion of PALB2 or BRCA1 reduced the RA-induced activation to 10 fold for each gene (Figure 4C). Importantly, depletion of retinoic acid receptor (RARa) diminished the recruitment of BRCA1, PALB2 and RNAPII following RA treatment concomitant with decreased RA-responsiveness (Figure 4D). These results point to a role for RARa in recruitment of BRCA proteins .

To assess the breadth of action of PALB2 and BRCA1 in RA responsiveness, we treated MCF7 cells with RA and performed a gene expression profiling using microarrays. Importantly, depletion of BRCA1 and PALB2 in three independent experiments blunted the responsiveness of nearly all RA responsive genes (248 genes), underscoring the importance of these proteins in RA signaling (Figure 4E). In addition to genes with known roles in RA mediated apoptosis signaling, many genes involved in oxidative stress and interferon signaling lost their responsiveness to RA following depletion of BRCA1 and PALB2 (Figure 4F).

RA acid induces growth suppression in a wide range of adult cells, including breast cancer cells (del Rincon et al, 2003; Hua et al, 2009). We tested the hypothesis that depletion of BRCA1 and PALB2, which resulted in impaired transcriptional response to RA, may also diminish the RA-mediated growth suppression using MCF7 cells. Indeed, depletion of BRCA1 or PALB2 resulted in a significant decrease in the RA-mediated growth suppression (Figure 4G), consistent with their roles in transcriptional co-activation of RA-responsive genes.

Figure 4



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Pathways	p-value	ratio	genes
Retinoic acid Mediated Apoptosis Signaling	4.24E+00	8.82E-02	PARP10,PARP12,PARP9,IFNAR1,IRF1,PARP14
NRF2-mediated Oxidative Stress Response	1.36E+00	2.62E-02	SOD2,GPX2,DNAJB1,DNAJB9,ABCC4
Interferon Signaling	1.00E-07	0.21	IFIT3,OAS1,IFITM1,IFNGR1,IFI35,IRF9,PSMB8,IFNAR2,IFNAR1,IRF1
Acute Phase Response Signaling	6.64E+00	6.78E-02	C1R,ORM1/ORM2,SOD2,C3,C1S,CFB,CP,SAA2,FGB,LBP,SAA1,FGG
Hepatic Fibrosis / Hepatic Stellate Cell Activation	4.70E+00	6.12E-02	VEGFA,IL8,ICAM1,IGFBP3,CD14,IFNGR1,LBP,IFNAR2,IFNAR1
iNOS Signaling	3.99E+00	9.43E-02	CD14,IFNGR1,LBP,IRF1,IRAK2

Figure 4. (A) and (B) Recruitment of BRCA1, PALB2 and RNAPII at the transcription start site and the 3' end of HOXA1 and HOXA2 genes upon stimulation with 10 uM RA. Wild-type MCF7 cells were collected at 0h, 6h and 24h following stimulation and cross-linked in formaldehyde for ChIP analysis. The breast cancer proteins are loaded on chromatin at HOX loci over time, peaking at 24h, with recruitment kinetics similar to RNAPII.
(C) BRCA1 and PALB2 are required for proper activation of HOX genes. MCF7 cells were infected with lentiviral shRNAs against PALB2 and BRCA1. Stably transduced cells were stimulated with retinoic acid for 24h (RA +). The expression analysis of HOXA1 and HOXA2 expression was normalized against GUSB expression and is reported as a fold-increase over unstimulated cells (RA -) infected with a non-targeting construct (CTRL). HOXA1 activation is dampened by over 70% upon depletion of the breast cancer proteins ($p<0.001$ over three independent experiments); HOXA2 activation is also significantly reduced ($p<0.02$) after silencing of PALB2 (50%) and BRCA1 (65%). (D) Recruitment of BRCA1 and PALB2 at HOX genes is RARa dependent. Chromatin occupancy of RNAPII, BRCA1 and PALB2 was analyzed upon 6h of RA stimulation in MCF7 cells infected with lentiviral shRNAs against RARa. (E) Global RA transcriptional response is diminished following BRCA1 and PALB2 depletion. The induction of RA top-responding genes at 24h has been analyzed in MCF7 cells using gene expression arrays. The heat map representation covers 248 microarray probes upregulated by retinoic acid in normal conditions (CTRL shRNAs, expression fold change of t=24h over t=0h, log2(FoldChange)>0.4). The color scale represents the modified log2 ratio ("sweep" function R, scaled by row) between the induced (24h) and the basal state (0h) of every shRNA condition. Results from three independent experiments are shown. (F) The table lists some of the most significant pathways regulated by retinoic acid in MCF-7 cells, including RA-mediated apoptosis and NRF2-dependent oxidative stress response (data were obtained using Ingenuity Canonical Pathways analysis).
(G) PALB2 and BRCA1 depletion attenuates RA growth suppression. MCF7 cells stably transfected with shRNAs against BRCA1, PALB2 and CRTL were plated at subconfluent density and treated with 10 uM RA for five days. Data are reported as percent of viable cells compared to the untreated control. There is a significant difference between CTRL and BRCA1/PALB2 depleted cells at days 4 and 5 ($p<0.05$).

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

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

_____Yes
__x___No

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

_____Yes
__x___No

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

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

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

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

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

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

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

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

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

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

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

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

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

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

20. Articles Submitted to Peer-Reviewed Publications.

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

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

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

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

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Genome-wide analysis reveals a role for BRCA1 and PALB2 in transcriptional co-activation	Gardini, A., Baillat, D., Cesaroni, M, and Shiekhattar, R.	The EMBO Journal	May 2013	☐Submitted ☐Accepted X 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 are planning to investigate the transcriptional activity of BRCA1 during TNF-alpha and RA activation in the context of embryonic stem cells, using the wild type BRCA1 as well as point mutations that have been found in BRCA1 families

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.

None

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.

None

23. Inventions, Patents and Commercial Development Opportunities.

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

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

If yes, indicate date patent was filed:

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

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

If yes, how many licenses were granted?_____

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

If yes, describe the commercial development activities:

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

Yes_____ No____x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME **SHIEKHATTAR, Ramin**	POSITION TITLE **Professor**
eRA Commons Username: **RAMIN_SHIEKHATTAR**	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Kansas, Lawrence, KS	B.S.	04/84	Chemistry
University of Kansas, Lawrence, KS	Ph.D.	04/89	Biochemistry

A. PERSONAL STATEMENT

We are interested in understanding the mechanisms by which protein-coding genes are regulated and how disruption of such regulatory mechanisms may lead to neoplasia. Recent evidence has implicated changes in chromatin structure as an important mechanism in gene regulation. Moreover, besides a classic role for proteins in mediating transcriptional and post-transcriptional effects, it has become clear that non-coding RNAs play an integral role in regulation through fine-tuning gene expression patterns during development and differentiation. We believe that this “signaling by RNA” is an emerging field of research that will uncover novel pathways in genome organization and regulation. In the past few years my laboratory has employed a biochemical approach to uncover a number of key factors that mediate transcriptional regulation through modulation of chromatin structure, or post-transcriptional silencing via non-coding RNAs. We will continue our studies through a detailed structure/function analysis of these factors in regulation of transcription and extend our experiments to elucidate the role these factors in the genesis of cancer.

B. POSITIONS AND HONORS

Research Experience:

1989-1993 Postdoctoral Fellow, Division of Behavioral Neurobiology, Department of Mental Health Sciences, Hahnemann University, Philadelphia, PA
1993-1997 Postdoctoral Fellow, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ
1997-2000 Assistant Professor, The Wistar Institute, Philadelphia, PA
2001-2005 Associate Professor, The Wistar Institute, Philadelphia, PA
2007-2009 Professor, Center for Genomic Regulation, Barcelona, Spain
2006-2014 Professor, The Wistar Institute, Philadelphia, PA
2014-present Professor, University of Miami, Miami, FL

Honors and Awards:

1993-1996 National Institute of Health National Research Service Award
1997-1998 American Cancer Society Institutional Research Grant
1999-2001 Scholar of V Foundation for Cancer Research
1998-2001 W.W. Smith Charitable Trust
2000-2004 Breast cancer Research Foundation
2001-2004 American Cancer Society

2006-2008 The Mather Foundation
2004-2005 BioAdvance

C. SELECTED PEER-REVIEWED PUBLICATIONS

1. Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F, **Shiekhattar R**. 2000. BRCA1 is associated with a human SWI/SNF-related complex: Linking Chromatin Remodeling to Breast Cancer. Cell 102:257-256. PMID: 10943845.
2. Marmorstein LY, Kinev AV, Chan GK, Bochar DA, Beniya H, Epstein JA, Yen TJ, **Shiekhattar R**. 2001. A Human BRCA2 Complex Containing a Structural DNA-binding Component Influences Cell Cycle Progression. Cell 104:247-257. PMID: 11207365.
3. Hakimi M-A, Bochar DA, Schmiesing JA Jr, Dong Y, Barak OG, Speicher DW, Yokomori K, **Shiekhattar R**. 2002. A chromatin remodeling complex that loads cohesin onto human chromosomes. Nature 418:994-998. PMID: 12198550.
4. Dong Y, Hakimi M-A, Chen X, Kumaraswamy E, Cooch NS, Godwin AK, **Shiekhattar R**. 2003. Regulation of BRCC, a Holoenzyme Complex Containing BRCA1 and BRCA2, by a Signalosome-like Subunit and its Role in DNA Repair. Mol Cell 12:1087-1099. PMID: 14636569.
5. Gregory RI, Yan K, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, **Shiekhattar R**. 2004. The microprocessor complex mediates the genesis of miRNA. Nature 432:235-240. PMID: 15531877.
6. Chendrimada T, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, **Shiekhattar R**. 2005. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436:740-744. PMCID: PMC2944926.
7. Lee MG, Wynder C, Cooch N, **Shiekhattar R**. 2005. An essential role for CoREST in nucleosomal histone H3 lysine 4 demethylation. Nature 437:432-435. PMID: 16079794.
8. Baillat D, Hakimi MA, Naar A, Shilatifard A, Cooch N, **Shiekhattar R**. 2005. Integrator, a multiprotein mediator of small nuclear RNA processing associates with the C-terminal of RNA polymerase II. Cell 123:256-276. PMID: 16239144.
9. Wynder C, Lee M, Hakimi M-A, Epstein J, **Shiekhattar R**. 2005. Recruitment of MLL by HMG-domain protein iBRAF promotes neural differentiation. Nat Cell Biol 7:1113-1117. PMID: 16227968.
10. Gregory RI, Chendrimada T, Cooch N, **Shiekhattar R**. 2005. Human RISC Couples MicroRNA biogenesis and posttranscriptional gene silencing. Cell 123:631-640. PMID: 16271387.
11. Lee MG, Villa R, Trojer P, Norman J, Yan KP, Reinberg D, Di Croce L, **Shiekhattar R**. 2007. Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. Science 318:447-450. PMID: 17761849.
12. Lee MG, Norman J, Shilatifard A, **Shiekhattar R**. 2007. Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein. Cell 128:877-887. PMID: 17320162.
13. Kumaraswamy E, **Shiekhattar R**. 2007. BACH1 is a component of a distinct BRCA1/BRCA2-containingcomplex involved in S phase progression. Mol Cell Bio 27:6733-6741. PMCID: PMC2099226.
14. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Busscotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Roderic G, **Shiekhattar R**. 2010. Long non-coding RNAs with enhancer-like function in human cells. Cell 143:46-58. PMID: 20887892.
15. Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA, **Shiekhattar R**. 2013. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. Nature 494:497-501. PMID: 23417068.