AWARD NUMBER: W81XWH-14-1-0238

TITLE: Targeting Histone Abnormality in Triple-Negative Breast Cancer

PRINCIPAL INVESTIGATOR: Nancy E. Davidson, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh, Pittsburgh, PA 15213

REPORT DATE: August 2016

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

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### Targeting Histone Abnormality in Triple-Negative Breast Cancer

#### Increasing lines of evidence showed that epigenetic regulation of TNBC proliferation and gene expression could provide novel targets for molecularly directed therapies for this devastating disease. In the first year of this award, our team collaborated with Dr. Huang's lab to demonstrate the coordinated overexpression of HDAC5 and LSD1 proteins in human primary breast tumor specimens. By using *in vitro* and *in vivo* models, we identified that sulforaphane (SFN), a natural bioactive HDAC inhibitor, destabilized LSD1 protein through downregulation of HDAC5 transcription, and combined use of SFN with a potent LSD1 inhibitor HCI-2509 significantly enhanced antineoplastic efficacy of SFN in MDA-MB-231 xenografts in mice. On the basis of these novel findings, we carried out microarray assays to address the global effect of HDAC5-LSD1 axis on gene expression in TNBC cells. This study successfully identified a subset of genes including a group of tumor suppressor genes whose expression was regulated by HDAC5-LSD1 signaling. During this funding period, our lab also explored the molecular mechanism of SFN induced suppression of HDAC5 transcription and performed the histological and pathological examinations of tissues, and interpretation of the results generated from *in vivo* studies. The collaborative studies between two labs have resulted in publication of a research article in *Oncogene*.
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1. INTRODUCTION

Emerging data indicate that epigenetic regulation of TNBC proliferation and metastasis may provide novel targets for molecularly directed therapies against this disease. Epigenetic changes include DNA methylation and modifications of the histone proteins that can switch genes on and off. The stepwise epigenetic changes have been implicated in transformation of normal breast epithelial cells into TNBC. The dysregulated epigenetic changes in TNBC frequently lead to loss of expression of important growth regulatory genes such as tumor suppressor genes (TSG) which facilitate malignant progression of this disease. Histone deacetylase 5 (HDAC5) and lysine specific demethylase 1 (LSD1) are two key epigenetic modifiers controlling chromatin levels of histone acetylation and methylation. In the first year of this award, we demonstrated that enhanced interaction between HDAC5 and LSD1 stabilizes LSD1 protein that in turn promotes tumor growth and metastasis of TNBC cells. We have also successfully identified that sulforaphane (SFN), a natural HDAC inhibitor, suppresses HDAC5 expression that in turn destabilizes LSD1 protein. Our preclinical data strongly suggest that targeting the HDAC5-LSD1 pathway by SFN in combination with a potent LSD1 inhibitor, HCI-2509, may represent a novel and effective approach for TNBC treatment. In the current funding year, in collaboration with Dr. Yi Huang’s lab, we performed genome-wide gene expression analysis in MDA-MB-231 cells with stable knockdown of HDAC5 or LSD1 to define a comprehensive profile of genes whose expression is associated with the HDAC5 and LSD1 signaling pathways. The bioinformatic analysis revealed a subset of genes including a group of TSG whose expression was regulated by the HDAC5-LSD1 axis. We also explored the molecular mechanism of SFN induced suppression of HDAC5 transcription and performed the histological and pathological examinations of tissues, and interpretation of the results generated from in vivo studies. The collaborative study between the two PIs has led to a paper recently published in Oncogene (2016 May 23, Epub ahead of print, PMID: 27212032).

2. KEYWORDS

Triple negative breast cancer, HDAC5, LSD1, sulforaphane, HCI-2509, combination therapy, microarray study

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

The major goal for the research award is to continue to understand the role of crosstalk between LSD1 and HDAC5 in promoting TNBC growth and metastasis, and seek for a novel therapeutic approach to target aberrant crosstalk between HDAC5 and LSD1 for poorly differentiated and aggressive TNBC. These goals are addressed with the close collaboration between two PIs through the following specific aims:

i. Delineate the molecular basis by which inhibition of LSD1 promotes HDACi-induced apoptosis through reactivation of aberrantly silenced tumor suppressor genes.

ii. Elucidate the role of LSD1 in HDACi therapy and chemoprevention of TNBC in animal models.

iii. Evaluate therapeutic effects of combination strategies in patient-derived xenografts (PDXs).

We anticipate that the novel results obtained from these proposed studies would address an unmet need to develop novel methods to determine which epigenetic changes contribute directly to TNBC development and decipher through in vitro and in vivo models how to apply the novel epigenetic reagents in most favorable combination strategy. The information derived from these studies will likely validate if HDAC5-LSD1 axis has potential to serve as novel therapeutic biomarkers to predict or indicate the response to epigenetic therapy in TNBC.

b. What was accomplished under these goals?
<table>
<thead>
<tr>
<th>Proposed Aims</th>
<th>Accomplishment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Aim 1:</strong> Delineate the molecular basis by which inhibition of LSD1 promotes HDACi-induced apoptosis through reactivation of aberrantly silenced tumor suppressor genes.</td>
<td>The Davidson team used microarray technology to investigate the alteration of genome-wide gene expression in MDA-MB-231 cells with stable knockdown of HDAC5 by shRNA. With the assistance of UPCI biostatisticians, her team evaluated the comprehensive gene expression profile affected by HDAC5-KD, and compared the results with gene expression profile changes caused by LSD1-KD. The goal of this study is to identify the unique subset of genes whose expression is associated with HDAC5-LSD1 signaling pathway.</td>
</tr>
<tr>
<td><strong>Major Task 3:</strong> Investigate genome-wide epigenetic gene silencing caused by crosstalk between LSD1 and HDACs. (Month 18-24)</td>
<td>To define a comprehensive profile of genes whose expression is associated with HDAC5 and LSD1 signaling pathways, we performed genome-wide gene expression analysis in MDA-MB-231 cells with stable knockdown of HDAC5 or LSD1 by shRNA. Microarray studies were performed at the UPCI Genomics Shared Facility. The array study was performed using Affymetrix Gene Chip U133A 2.0 array platform, which contains 20,928 probes representing all functionally characterized genes in the human genome. Raw intensity data were normalized by the Robust Multi-array Average (RMA). Student’s t-tests were performed in R/Bioconductor to identify differentially expressed genes. Probe level expression values were condensed to individual genes by choosing the individual probe with the lowest q-value. The list of genes significantly differentially expressed (FDR &lt;0.10) was first filtered to remove probes with mean signal intensities of less than 50 units in both sample groups and to remove probes that are not unique to a single transcript or common among transcripts from the same gene (_at or _s_at). Venn diagram was generated using the BioVenn-web application. Data were analyzed through the use of QIAGEN's Ingenuity® Pathway Analysis (Redwood City, CA). Heatmap was generated using TM4 system (Boston, MA). We identified 2477 and 1726 genes whose expression was significantly changed by inhibition of HDAC5 and LSD1, respectively. Strikingly, over 30% of genes in each group were changed by HDAC5-KD or LSD1-KD (Fig. 1A). This result reflects a comprehensive genome wide cooperative effect of HDAC5 and LSD1 on gene expression patterns. Ingenuity pathway analysis showed that these commonly targeted genes have important roles in a wide range of cellular functions in breast cancer (Fig.</td>
</tr>
</tbody>
</table>
The in-depth bioinformatic analysis revealed a subset of genes including a group of TSG whose expression was reactivated by HDAC5-KD or LSD1-KD (Fig. 1C). These genes have the potential to play an important role in breast tumorigenesis and therapeutic response.

Figure 1. Genome-wide microarray analysis of target genes of HDAC5-LSD1 axis. (A) Diagrams of commonly regulated genes by HDAC5-KD and LSD-KD in MDA-MB-231 cells were shown. (B) Functional analysis of genes whose expression is modulated by HDAC5-LSD1 axis. The bar graphs were identified by Ingenuity Pathway Analysis (IPA). (C) List of representative target genes of HDAC5-LSD1.

### Proposed Aims

#### Specific Aim 2: Elucidate the role of LSD1 in HDACi therapy and chemoprevention of TNBC in animal models.

#### Major Task 4: Evaluate in vivo therapeutic effects of combination strategies using LSD1 inhibitors and HDACi in different subtypes of breast tumors.

#### Subtask 2: Examine the in vivo effects of LSD1/HDAC signaling on tumorigenic transformation of MCF-10A cells (Month 8-1B).

In the first funding year, the Davidson lab successfully carried out the animal experiments and demonstrated that natural bioactive product, sulforaphane, in combination with a potent LSD1 inhibitor, HCI-2509, exhibited statistically significant enhanced inhibitory effect on MDA-MB-231 tumor growth in nude mice when compared with either drug alone. In the current funding period, the Davidson lab extended the tumor sample analysis, histological and pathological examinations of tissues, and interpretation of results generated from in vivo studies. In addition, her team carried out studies to investigate the mechanism underlying the regulation of SFN on HDAC5 transcriptional activity in breast cancer cells.

This study is being planned and we expect to start the experiment shortly.

<table>
<thead>
<tr>
<th>Proposed Aims</th>
<th>Accomplishment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Aim 2:</strong> Elucidate the role of LSD1 in HDACi therapy and chemoprevention of TNBC in animal models.</td>
<td>In the first funding year, the Davidson lab successfully carried out the animal experiments and demonstrated that natural bioactive product, sulforaphane, in combination with a potent LSD1 inhibitor, HCI-2509, exhibited statistically significant enhanced inhibitory effect on MDA-MB-231 tumor growth in nude mice when compared with either drug alone. In the current funding period, the Davidson lab extended the tumor sample analysis, histological and pathological examinations of tissues, and interpretation of results generated from in vivo studies. In addition, her team carried out studies to investigate the mechanism underlying the regulation of SFN on HDAC5 transcriptional activity in breast cancer cells.</td>
</tr>
<tr>
<td><strong>Major Task 4:</strong> Evaluate in vivo therapeutic effects of combination strategies using LSD1 inhibitors and HDACi in different subtypes of breast tumors.</td>
<td>This study is being planned and we expect to start the experiment shortly.</td>
</tr>
</tbody>
</table>
Subtask 3: Evaluate combination strategies using LSD1i and HDACi in different subtypes of breast tumors (Month 10-26).

The poly(ADP-ribose) polymerase (PARP-1) is a 113 kDa nuclear enzyme which can be cleaved in fragments of 89 and 24 kDa during apoptotic cell death. Thus this cleavage has become a useful hallmark of apoptosis. To determine if combined therapy using SFN and HCI-2509 promotes MDA-MB-231 xenograft tumor cell apoptosis, level of full length PARP-1 cleavage was measured. MDA-MB-231 cells were transplanted into the mammary gland of nude mice. Seven days after implantation, sulforaphane (50 mg/kg), HCI-2509 (30 mg/kg), combination (SFN 50mg/kg+HCI-2509 30mg/kg) or vehicle (DMSO) were delivered via i.p. injection for 5 day/week x 4 weeks. Fifty micrograms of whole cell protein (n=9 for each group) per lane were analyzed for expression of full length PARP-1 and β-actin as a loading control. A quantitative analysis showed the combination induced significantly more PARP-1 cleavage in MDA-MB-231 xenograft tumor cells evidenced by significantly reduced level of full length PARP-1 compared with the single agent tumors (Fig. 2A).

To further evaluate possible undesirable cytotoxic effects in treated animals, we performed hematoxylin-eosin (H&E) sections of animal livers and kidneys; these showed no apparent morphological changes in the tissues treated with SFN or HCI-2509 alone, or in combination (Fig. 2B). Collectively, these results indicate that SFN effectively inhibits growth of breast tumor in vivo but is more effective when used in combination with an LSD1 inhibitor; neither the single agents nor the combination led to histological evidence of liver or kidney toxicity.

Other reportable results

In first funding year, we demonstrated that sulforaphane (SFN), a natural HDAC inhibitor found in cruciferous vegetables, significantly inhibited mRNA expression of HDAC5 in MDA-MB-231 cells. In this award period, we compared the effect of SFN on expression of HDAC5, LSD1 and USP28 with some other HDACi that included hydroxamic acid derivatives SAHA (Vorinostat), TSA (Trichostatin A), LBH-589 (Panobinostat), and PXD-101 (Belinostat), a benzamide analog MS-275 (Entinostat), and a selective class II (IIa) histone deacetylase (HDAC II) inhibitor MC-1568. In MDA-MB-231 cells, treatment with most of the compounds led to significant increase of HDAC5.
mRNA expression with marginal effect on mRNA levels of LSD1 and USP28. However, among these HDACIs, SFN significantly inhibited mRNA expression of HDAC5 without altering mRNA levels of LSD1 and USP28 (Fig. 3). The above studies further confirm that SFN acts as a unique HDACI in suppression of HDAC5 mRNA expression in human breast cancer cells.

The effects of selected HDAC inhibitors on HDAC5 transcription were studied using the element of -635 to -893 bp of HDAC5 promoter with full length construct (-1251 to + 666 bp) as control. This was based on results from the Huang lab that this element might play an essential role in mediating transcriptional activity of the HDAC5. Cells/per well were seeded into 24 well plate, 250 ng plasmids pGL2-Enhancer or the constructs of pGL2-Enhancer-HDAC5 promoter was transiently co-transfected with 25 ng pRL-TK (Promega) into cells using Lipofectamine 3000 (Invitrogen), separately. 48 h post-transfection, cells were harvested and lysed in buffer from Dual-luciferase assay kit (Promega), with luciferase activity measured on a GLOMAX® 20/20 luminometer (Promega). Luciferase values (relative light units) were normalized to the Renilla luciferase activity and expressed as the fold change relative to pGL2-Enhancer transfected wells. Treatment with SFN significantly inhibited the reporter gene activity of the full length and -635 to -839 bp constructs in MDA-MB-231 cells. In contrast, exposure of MDA-MB-231 cells to SAHA led to increased reporter gene activity on the full length and -635 to -839 constructs (Fig. 4A and 4B). Similar results were observed in another breast cancer line MDA-MB-468 cells (data not shown). These data shed light on the mechanisms underlying transcriptional regulation of HDAC5 promoter activity in breast cancer cells and identify -635 to -839 as an important regulatory element for SFN at HDAC5 promoter. These results also suggest that upregulation of HDAC5 expression by other HDACs such as SAHA might act as a self-defense mechanism against antitumor effect of HDACIs and may contribute to refractoriness to SAHA therapy in breast cancer.
Figure 2. Effect of combination therapy on full length PARP-1 expression and histological evaluation of animal tissues (A) Whole cell proteins were extracted from tumor samples of MDA-MB-231 xenografts treated with vehicle, HCI-2509, SFN or combination for 4 weeks (n=9 from each group). Quantitative immunoblotting was used to determine the expression of full length PARP-1 protein. β-actin was used as control. Data were represented as the mean ± s.d of three independent experiments. The quantitative variables were analyzed by the two-tailed Student’s t-test. P-value<0.05 was considered statistically significant for all tests. (B) Histological analysis of liver and kidney of xenograft mice treated with vehicle, HCI-2509, SFN or combination for 4 weeks (n=3 from each group). After the mice were euthanized, the organs were excised and stained with hematoxylin and eosin (H & E). The images of each group are depicted at an original magnification of 100×.

Figure 3. Effect of HDAC inhibitors on expression of HDAC5, LSD1 and USP28 in human breast cancer cells. After MDA-MB-231 cells were exposed to HDAC inhibitors for 24 h, cellular mRNA expression of HDAC5, LSD1 and USP28 was quantitatively measured by real-time PCR. Data were represented as the mean ± s.d of three independent experiments. The quantitative variables between HDACi treated sample and vehicle treated sample were analyzed by the two-tailed Student’s t-test. P-value<0.05 was considered statistically significant for all tests.
b. What opportunities for training and professional development has the project provided?

The postdoc fellow who participates in this project has many opportunities for training and professional development. Travel costs for postdoc fellow to attend the local or national cancer conferences were supported by the project. There are numerous training sessions at the conferences and seminars inside or outside of our institute. Each of our fellows has many opportunities to present their work to their colleagues at these conferences. In addition, this project provided support and an excellent training opportunity for visiting and rotating scholars/students in the laboratory.

c. How were the results disseminated to communities of interest?

This work has been published in *Oncogene*: Cao C, Vasilatos SN, Bhargava R, Fine J, Oesterreich S, Davidson NE, Huang Y. Functional interaction of histone deacetylase 5 (HDAC5) and lysine-specific demethylase 1 (LSD1) promotes breast cancer progression. Oncogene, 2016. PMID: 27212032

d. What do you plan to do during the next reporting period to accomplish the goals?

As summarized in Figure 5, we have demonstrated that LSD1 protein stability is promoted by HDAC5 through the LSD1 associated ubiquitin-proteasome system, confirming that the regulation of LSD1 by HDAC5 is a posttranslational event. Our novel findings also provide supportive evidence that an orchestrated interaction between HDAC5 and LSD1 is a critical epigenetic mechanism to suppress transcriptional activities of important tumor suppressor genes that may contribute to breast cancer development. During the next
reporting period, we plan to perform the following studies to accomplish the goals: (1) in collaboration with Dr. Huang’s team, we will further elucidate the mechanism of SFN-induced downregulation of HDAC5 transcription in breast cancer cells. Quantitative ChIP assays will be used to illustrate how SFN disrupts the DNA binding activity of the regulatory factors at Sulfuraphane Response Element (SRE). Furthermore, the cellular sensitivity to SFN will be measured in cells overexpressing the key factors associated with SRE and to determine whether SFN hinders TNBC growth through disruption of the normal transcriptional activity at SRE. (2) To study the mechanisms of how HDAC5 inhibition sensitizes TNBC cells to novel LSD1 inhibitors. We will address this question through the following studies: (a) we will utilize the MCF10A progression model to examine the effect of combination therapy on transformation of MCF10A cells and access whether combination therapy would more profoundly inhibit stem-cell-like traits of TNBC cells. (b) We will determine the potential synergistic effect of the combination treatment of SFN with novel LSD1 inhibitors on the expression of TSGs regulated by HDAC5-LSD1. (3) To further elucidate the in vivo function of HDAC5-LSD1 pathway in TNBC development and evaluate the effect of inhibition of HDAC5-LSD1 axis in TNBC therapy using murine models. We will test the in vivo effect of SFN on growth of TNBC cells overexpressing HDAC5 or LSD1. Moreover, animal work will be carried out to determine whether inhibition of the HDAC5-LSD1 axis effectively prevents the metastasis of TNBC in vivo.

4. IMPACT

(a) What was the impact on the development of the principal discipline(s) of the project? The proposed studies of this award addresses an unmet need to develop novel methods to define which epigenetic changes contribute directly to TNBC development and decipher through in vitro and in vivo models how to apply the novel epigenetic reagents in most favorable combination strategy. The information derived from these studies will likely validate if elements of the HDAC5-LSD1 axis have potential to serve as novel therapeutic biomarkers to predict response to epigenetic therapy in TNBC. Targeting HDAC5 inhibition with the natural product, sulforaphane, in combination with a newly developed potent LSD1 inhibitor, HCL-2509, showed superior antineoplastic activity both in vitro and in vivo. This proposal seeks to uncover how the HDAC5-LSD1 axis contributes to resistance to HDACi therapy in breast cancer. The information gained from this study could lead to validation and translation of our new strategy into future trials.

(b) What was the impact on other disciplines? Nothing to Report

(c) What was the impact on technology transfer? Nothing to Report

5. CHANGES/PROBLEMS

(a) Changes in approach and reasons for change

No major changes in approach have been made since the initiation of the award.

(b) Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

(c) Changes that had a significant impact on expenditures

Nothing to Report
(d) Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

6. PRODUCTS

(a) Publications, conference papers, and presentations

Research Article:


Conference Abstract:


(b) Website(s) or other Internet site(s) Nothing to Report

(c) Technologies or techniques Nothing to Report

(d) Inventions, patent applications, and/or licenses Nothing to Report

(e) Other Products Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a) Individuals have worked on the project

<table>
<thead>
<tr>
<th>Name:</th>
<th>Nancy Davidson</th>
<th>Yi Huang</th>
<th>Chunyu Cao</th>
<th>Shauna Vasilatos</th>
<th>Ye Qin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>Co-PI</td>
<td>Co-PI</td>
<td>Postdoc Fellow</td>
<td>Technician</td>
<td>Postdoc Fellow</td>
</tr>
<tr>
<td>Researcher Identifier (e.g. ORCID ID):</td>
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<td>N/A</td>
<td>N/A</td>
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<td>Nearest person month worked:</td>
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<td>6.0</td>
<td>9.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Oversaw epigenetic drug studies and animal experiments, and interpreted the results generated from combination</td>
<td>Designed and oversaw the studies to investigate the molecular mechanisms and biological</td>
<td>Studied molecular mechanisms by which LSD1 and HDAC5 interacted, and</td>
<td>Performed microarray studies and data analysis</td>
<td>Newly recruited postdoc fellow.</td>
</tr>
</tbody>
</table>
b) Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Chunyu Cao has completed his postdoctoral fellow training, and Dr. Ye Qin joined the laboratory to continue the work as a postdoctoral fellow.

c) What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Initiating PI, Dr. Yi Huang, will submit his annual report separately.

QUAD CHARTS: N/A

9. APPENDICES: updated curriculum vitae is attached
CURRICULUM VITAE
University of Pittsburgh
School of Medicine

BIOGRAPHICAL

Name: Nancy Ellen Davidson, MD
Address: 537 North Neville Street
         Pittsburgh, PA  15213
Birth Place: Denver, Colorado

Home Phone: 412-683-7178
Citizenship: United States

Business Address: University of Pittsburgh Cancer Institute
                 UPMC Cancer Pavilion
                 5150 Centre Avenue, Suite 500
                 Pittsburgh, PA  15232
Email Address: davidsonne@upmc.edu

Business Phone: 412-623-3205
Business Fax: 412-623-3210

EDUCATION and TRAINING

UNDERGRADUATE:
1971-1975 Wellesley College
       Wellesley, Massachusetts
         BA 1975 Molecular Biology

GRADUATE:
1975-1979 Harvard Medical School
       Boston, Massachusetts
         MD 1979 Medicine

POSTGRADUATE:
1979-1980 Hospital of the University of Pennsylvania
         Intern 1980 Internal Medicine
         Philadelphia, Pennsylvania

1980-1982 The Johns Hopkins Hospital
         Resident 1982 Internal Medicine
         Baltimore, Maryland

1982-1985 National Cancer Institute
         Medical Staff Fellow 1985
         National Institutes of Health
         Bethesda, Maryland
APPOINTMENTS and POSITIONS

ACADEMIC:

1985-1987  Medical Breast Cancer Section, 
            Medicine Branch, National Cancer Institute 
            Bethesda, Maryland

1985-1986  Uniformed Services 
            University of Health Sciences 
            Bethesda, Maryland

1986-1992  The Johns Hopkins University 
            Baltimore, Maryland

1986-1992  The Johns Hopkins University 
            Baltimore, Maryland

1995-2009  The Johns Hopkins University 
            Baltimore, Maryland

1999-2009  The Johns Hopkins University 
            Baltimore, Maryland

1986-2009  Johns Hopkins Hospital 
            Baltimore, Maryland

1994-2009  The Johns Hopkins Oncology Center 
            Baltimore, Maryland

1997-2009  The Johns Hopkins Bloomberg 
            School of Public Health 
            Baltimore, Maryland

2009-      The Johns Hopkins University 
            Baltimore, Maryland

2009-      University of Pittsburgh 
            Pittsburgh, PA

2009-      University of Pittsburgh 
            Pittsburgh, PA

            Guest Worker
            Research Assistant 
            Professor of Pharmacology
            Assistant Professor of Oncology 
            Associate Professor of Oncology
            Breast Cancer Research Chair 
            of Oncology
            Professor of Oncology
            Active Staff
            Director, Breast Cancer Program
            Joint Appointment in Department 
            of Biochemistry and Molecular Biology
            Adjunct Professor of Oncology
            Chief, 
            Division of Hematology/Oncology
            Director, 
            University of Pittsburgh Cancer Institute 
            Professor of Medicine and 
            Pharmacology and Chemical Biology 
            Associate Vice Chancellor for 
            Cancer Research 
            Hillman Professor of Oncology
CERTIFICATION AND LICENSURE

SPECIALTY CERTIFICATION:
Certifying Board                        Year
National Board of Medical Examiners     1980
American Board of Internal Medicine     1982
Medical Oncology                       1985

Medical or other Professional Licensure:
State of Maryland                       1982
Commonwealth of Pennsylvania            2009

MEMBERSHIPS in PROFESSIONAL and SCIENTIFIC SOCIETIES

<table>
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<tr>
<th>Organization</th>
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<tr>
<td><strong>American Society of Clinical Oncology</strong></td>
<td><strong>1985-present</strong></td>
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<tr>
<td>Member, Public Issues Committee</td>
<td>1992-1996</td>
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<td>Member, Award Selection Committee</td>
<td>1992-1996</td>
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<tr>
<td>Chair, Award Selection Committee</td>
<td>1994-1995</td>
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<tr>
<td>Member, Ad hoc Technology Assessment Committee</td>
<td>1993-1994</td>
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<tr>
<td>for Development of Growth Factor Clinical Practice Guidelines</td>
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<tr>
<td>Co-Chair, Breast Cancer Follow-up Testing Guidelines Expert Panel</td>
<td>1996- present</td>
</tr>
<tr>
<td>Member, Membership Committee</td>
<td>1997-1999</td>
</tr>
<tr>
<td>Member, Board of Directors</td>
<td>1996-1999</td>
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<tr>
<td>Member, Grants Selection Committee</td>
<td>1999-2002</td>
</tr>
<tr>
<td>Member, Task Force on Quality of Cancer Care</td>
<td>1999-2004</td>
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<tr>
<td>Member, Publications Committee</td>
<td>2004-2007</td>
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<td>Chair, Publications Committee</td>
<td>2005-2006</td>
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<tr>
<td>Member, Translational Research Task Force</td>
<td>2005-2006</td>
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<td>President-Elect, President, and Immediate Past President</td>
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<td>Member, Value in Cancer Care Task Force</td>
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<td>Chair, Special Awards Selection Committee</td>
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<td>Member, Translational Research Professorship Selection Committee</td>
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<tr>
<td>Government Relations Committee</td>
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<td><strong>American Association for Cancer Research</strong></td>
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<td>Member, Maryland Legislative Committee</td>
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<td>Member, Program Committee</td>
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<td>Co-Chair, Program Committee</td>
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<tr>
<td>Member, Clinical Cancer Research Committee</td>
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<td>Member, AACR-Richard and Hinda Rosenthal Foundation Award</td>
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<td>Prevention Research Conference</td>
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<tr>
<td>Member and Chair (2010-11), AACR Nominating Committee</td>
<td>2010-2012</td>
</tr>
<tr>
<td>Member, Continuing Medical Education Committee, (Chair 2015-2016)</td>
<td>2010-2016</td>
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<tr>
<td>Member, Education Committee</td>
<td>2012-2013</td>
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<tr>
<td>Member, Program Committee,</td>
<td>2011-2014</td>
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<tr>
<td>AACR-San Antonio Breast Cancer Symposium</td>
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<tr>
<td>President-Elect, President, and Immediate Past President</td>
<td>2015-2018</td>
</tr>
<tr>
<td><strong>Eastern Cooperative Oncology Group</strong></td>
<td>1987-present</td>
</tr>
<tr>
<td>Member, Breast Cancer Core Committee</td>
<td>1987-present</td>
</tr>
<tr>
<td>Chair, Breast Cancer Biology Committee</td>
<td>1992-1996</td>
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<tr>
<td>Co-Chair, Breast Cancer Committee</td>
<td>1992-1996</td>
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<tr>
<td>Chair - Breast Cancer Committee</td>
<td>1997-2002</td>
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<tr>
<td><strong>American College of Physicians</strong></td>
<td>2009-present</td>
</tr>
<tr>
<td>Member</td>
<td></td>
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<tr>
<td>Member, National Surgical Adjuvant Bowel and Breast Project</td>
<td>2010-present</td>
</tr>
<tr>
<td>Member, Board of Directors, Association of American Cancer Institute’s</td>
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<tr>
<td>Member, Association of American Physicians Council</td>
<td>2015-present</td>
</tr>
</tbody>
</table>
HONORS

Phi Beta Kappa 1974
Sigma X 1975
American Society of Clinical Oncology Young Investigator Award 1986-1987
Susan Komen Foundation Award 1987-1988
American Cancer Society Clinical Oncology Career Development Award 1988-1991
Merck Clinician Scientist Award 1989-1990
Breast Cancer Research Chair in Oncology, Johns Hopkins 1995-2009
ACS Research Award, American Cancer Society - Maryland Division 1998
Brinker International Award for Breast Cancer Research 1999
Wellesley College Alumnae Achievement Award 2000
William L. McGuire Memorial Lectureship, 24th 2001
Annual San Antonio Breast Cancer Symposium
Avon Foundation Medical Advancement Award 2003
President, American Society of Clinical Oncology 2007 - 2008
7th Rosalind E. Franklin Award for Women in Science, 2008
National Cancer Institute
11th American Association for Cancer Research-Women in Cancer Research Charlotte Friend Award
Johns Hopkins University Alumni Association Distinguished Alumna Award 2009
American Society of Clinical Oncology Gianni Bonadonna Breast Cancer Award 2010
Association of American Physicians 2010
National Academy of Medicine (formerly the Institute of Medicine) 2011
Pennsylvania Breast Cancer Coalition Potamkin Award 2012
Distinguished Professor of Medicine, University of Pittsburgh 2013
Thomson Reuters Highly Cited Researchers 2014, 2015
The Johns Hopkins Women’s Medical Alumnae Assoc. Hall of Fame 2015
Johns Hopkins University Society of Scholars 2016
Fellow, American College of Physicians 2016

PUBLICATIONS

Refereed Articles:

2. Kensler TW, Busby WF, Davidson NE, and Wogan GN. Effect of hepatocarcinogens on the binding of glucocorticoid-receptor complex in rat liver nuclei. Cancer Res. 36:4647-51, 1976. PMID 1000507
22. Rowley SD, Piantadosi S, Marcellus DC, Jones RJ, Davidson NE, Davis JM, Kennedy J, Wiley JM, Wingard J, Yeager AM and Santos GW. Analysis of factors predicting speed of hematologic recovery after transplantation with 4 hydroperoxycyclophosphamide-purged autologous bone marrow grafts. Bone Marrow Trans. 7:183-91, 1991. PMID 2059755
34. Davidson NE, Mank AR, Prestigiacomo LJ, Bergeron RJ and Casero RA. Growth inhibition of hormone-responsive and -resistant human breast cancer cell lines by N\textsuperscript{1},N\textsuperscript{12}-bis(ethyl) spermine. Cancer Res. 53:2071-5, 1993. PMID 8481909


61. Issa J-P, Zehnbauer BA, Civin CI, Collector M, Sharkis SJ, Davidson NE, Kaufmann SH and Baylin SB. The estrogen receptor CpG island is methylated in most hematopoietic neoplasms. Cancer Res. 56:973-77, 1996. PMID 8640788


75. Ferguson AT, Lapidus RG and Davidson NE. Demethylation of the progesterone receptor CpG island is not required for progesterone receptor gene expression. Oncogene. 17:577-83, 1998. PMID 9704923


78. Hahm HA and Davidson NE. Apoptosis in the mammary gland and breast cancer. Endocrine-Related Cancer. 5:199-211, 1998.


102. Dees EC and Davidson NE. Ovarian ablation as adjuvant therapy for breast cancer. Seminars in Oncology. 28:322-33, 2001. PMID 11498826


120. Emens LA and Davidson NE. The follow-up of breast cancer. Semin Oncol. 338-348, 2003. PMID 12870135


128. Gabrielson E, Tully E, Hacker A, Pegg A, Davidson N and Casero R. Induction of spermidine/spermine N1-acetyltransferase (SSAT) in breast cancer tissues treated with the
polyamine analogue N1-N11-diethylnorspermine. Cancer Chemotherapy Pharmacol. 54:122-6, 2004. PMID 15138709


130. Prowell TM and Davidson NE. What is the role of ovarian ablation in the management of primary and metastatic breast cancer today? The Oncologist. 9:507-17, 2004. PMID 15477635


137. Huang Y, Pledgie A, Casero RA and Davidson NE. Molecular mechanisms of polyamine analogues in cancer cells, Anti-Cancer Drugs. 16: 229-41, 2005. PMID 15711175


166. Visvanathan K, Santor D, Ali SZ, Brewster A, Arnold A, Armstrong DK, Davidson NE, Helzlsouer KJ. The reliability of nipple aspirate and ductal lavage in women at increased


175. Bao T, Davidson NE. How we maintain bone health in early stage breast cancer patients on aromatase inhibitors, J Oncology Practice. 3:323-5, 2007. PMC2793755


paclitaxel with or without trastuzumab in the North Central Cancer Treatment Group N9831 adjuvant breast cancer trial. J Clin Oncol. 26:1231-8, 2008. PMC 4048960, NIHMS 589003


endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol. 28:3784-96, 2010 PMID 20625130


217. Davidson, NE. HER2-targeted therapies: how far we’ve come—and where we’re headed. Oncology. 25(5):425-6, 2011. PMID 21710840

218. Davidson, NE. Retrospective on the last quarter-century in medical oncology. Oncology. 25(5):396, 2011. PMID 21710831


254. Davidson NE. Fifteen years of anti-HER2 therapy. Oncology. 27(3):151, 2013. PMID 23687781


265. Jankowitz RC, Davidson NE. Adjuvant endocrine therapy for breast cancer: how long is long enough? Oncology. 27(12):1210-6, 2013. PMID: 24624537


Editorials:


Articles in the Lay Press:

Book Reviews:

Books Edited:

Book Chapters:


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PROFESSIONAL ACTIVITIES

**Teaching:**

1988-2005 Lecturer, Pathophysiology Course for 2nd Year Medical Students

1991 Medical Student Advisor

1996-2009 Lecturer, Fundamentals of Clinical Oncology for Public Health Practitioners

1997-2009 Lecturer, Topics in Molecular Endocrinology, School of Public Health
2004-2009  Lecturer, Pathophysiology of Disease, Cellular and Molecular Medicine
2010-present Lecturer, Cancer Biology and Therapy
2013-present ILS Neoplasia & Neoplastic Diseases

Mentoring:
Postdoctoral Fellows – Laboratory  Current Position
1988-1990  M. John Kennedy, MD  Consultant, St. James Hospital, Dublin, Ireland
1989-1992  Deborah K. Armstrong, MD  Professor of Oncology, Johns Hopkins
1991-1993  Yvonne L. Ottaviano, MD  Private Practice, Baltimore, MD
1993-1996  Diane McCloskey, PhD  Associate Professor of Cellular & Molecular Physiology, Penn State, Hershey, PA
1993-1997  Rena Lapidus, PhD  Director, Translational Core Laboratory
1993-1998  Anne Ferguson, PhD  Associate Professor, University of Maryland
1994-1995  Christian Jackisch, MD  Private Practice, Baltimore, MD
1996-1999  Hillary Hahm, MD, PhD  Director of the National Cancer Policy Forum
1997-1999  Sharyl Nass, PhD  Institute of Medicine, National Academy of Medicine, Washington, DC
1998-2001  Xiaowei Yang, MD, PhD  Staff Scientist, National Cancer Institute
1999-2001  Valerie Dunn, MD  Private Practice, Rochester, NY
2000-2001  Lan Yan, MD, PhD  Staff Scientist, Amgen, Thousand Oaks, CA
2001-2006  Yi Huang, MD, PhD  Assistant Professor, University of Pittsburgh, Pittsburgh, PA
2001-2004  Judith C. Keen, PhD  Director of Scientific Affairs at the American Society for Radiation Oncology (ASTRO)
2002-2003  Dipali Sharma, PhD  Associate Professor of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD
2004-2008  Qun Zhou, MD, PhD  Associate Professor of Biochemistry and Molecular Biology, University of Maryland Medical School
2005-2006  Allison Tracy, PhD  Lecturer of Chemistry and Biochemistry, UMBC
2006-2009  Qingsong Zhu, PhD  Chief Operating Officer, InSilico Medicine, Inc., Baltimore, MD
2006-2009  Madhavi Billam, PhD  Senior Toxicologist, L’Oreal USA & RI
2011-2013  Tiffany Katz, PhD  Postdoctoral Research Associate, Texas A&M University
2014-present  Nilgun Tasdemir, PhD  Postdoctoral Fellow, University of Pittsburgh
2015-present  Lin Chen  Pre-doctoral Student

Doctoral Students
2000-2005  Julie Blum, PhD  Clinical Content Manager, MED-IQ
2001-2005  Allison Pledgie, PhD  Senior Lecturer in Chemistry and Biochemistry at University of Maryland Baltimore County (UMBC)
2004-2010  Abigail Witt, PhD  Postdoctoral Fellow, University of Miami School of Medicine
2005-2010 Talmesha Richards, PhD
Chief Academic and Diversity Officer at
STEMconnector

2006-2010 Patrick Shaw, PhD
Chief, Pathogen Detection Lab. USA Public Health Command Region-Pacific Camp Zama, Japan

Graduate Training Programs

1997-2011 Biochemistry and Molecular Biology, Hopkins Bloomberg School of Public Health (adjunct 2009-2011)
1999-2013 Cellular and Molecular Medicine, Hopkins School of Medicine (adjunct 2009-2013)

RESEARCH:

1. Current Grant Support

<table>
<thead>
<tr>
<th>Grant ID</th>
<th>Agency</th>
<th>Description</th>
<th>PI</th>
<th>Funding Period</th>
<th>Funding Amount</th>
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<tr>
<td>NIH P30CA047904</td>
<td>Cancer Center Support Grant</td>
<td>50%</td>
<td>2009-2020</td>
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<tr>
<td>TBCRC-JHU PO200066781</td>
<td>Translational Breast Cancer Research Consortium and the Komen Foundation.</td>
<td>2%</td>
<td>2009-2021</td>
<td>$6,786</td>
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<tr>
<td>TBCRC-JHU 2000805196</td>
<td>Translational Breast Cancer Research Consortium and the Avon Foundation.</td>
<td>1%</td>
<td>2009-2021</td>
<td>$6,080</td>
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<td>DOD W81XWH-14-1-0237</td>
<td>Targeting Histone Abnormality in Triple-Negative Breast Cancer</td>
<td>10%</td>
<td>2014-2017</td>
<td>$112,480</td>
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<td>U10 CA180844</td>
<td>NCI NCTN-Network Lead Academic Site at UPCI</td>
<td>5%</td>
<td>2014-2019</td>
<td>$11,255</td>
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<td>Davidson, Co-PI</td>
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1a. Prior Grant Support

Grants Awarded as Principal Investigator

1986-1987 American Society of Clinical Oncology Young Investigator Award, "Isolation of estrogen-induced genes from human breast cancer."

epidermal growth factor receptor and estrogen receptor in breast cancer."
1987-1989 American Cancer Society Maryland Division. "The role of epidermal growth
factor and its receptor in breast cancer."
1987-1988 Susan G. Komen Foundation. "The role of epidermal growth factor and
its receptor in human breast cancer."
1989-1995 NIH Grant R29 CA 49634. "Epidermal growth factor receptor in human
breast cancer."
1989-1990 Phil N. Allen Charitable Trust Grant. "Novel approaches to hormone-unresponsive breast
cancers."
1989-1990 Merck Clinician Scientist Award, Johns Hopkins University School of Medicine.
1990-1991 Johns Hopkins University School of Medicine Institutional Research Grant,
"Elimination of breast cancer cells from human bone marrow by counterflow
centrifugal elutriation".
in the germ-line of young women with breast cancer".
1992-1994 NIH Grant P30 CA06973 pilot. "DNA methylation and estrogen receptor expression in
human breast cancer cells"
1994-1998 NIH Grant R21 CA/ES 66204 "Development of a breast cancer program at
Johns Hopkins".
1994-1995 NIH Grant 5P50 CA-58236 pilot. "Effects of polyamine analogues on
growth of human prostatic cancer cells"
1995-1999 NIH Grant 1 U01 CA66084. "New therapeutic approaches for breast cancer".
1995-1998 American Cancer Society BE-237 "Methylation of steroid receptors in
human breast cancer".
of estrogen receptor in breast cancer.”
1998-2004 NIH Grant R01 CA78352 “DNA methylation as a determinant of hormone
resistant breast cancer.”
actions of a novel polyamine analog against breast cancer”
2001-2009 Avon Foundation
2001-2005 Susan G. Komen Foundation Postdoctoral Fellowship
2003-2005 Susan G. Komen Foundation Predoctoral Fellowship
2000-2009 American Breast Cancer Foundation
2006-2008 Susan G. Komen Foundation, BCTR 65706, “Polyamine analogues as
novel anti-estrogen receptor alpha agents
2007-2009 Lee Jeans Translational Breast Cancer Research Program (with
Entertainment Industry Foundation)
2008-2011 Susan G. Komen for the Cure, KG080923, Inhibition of lysine specific
demethylase 1 (LSD1) as a strategy for re-expression of epigenetically silenced
genes in breast cancer Robert Casero, Jr. and Nancy E. Davidson (Co-PIs)
2000-2013 NIH P50 CA88843. SPORE in Breast Cancer (co-PI of University of Pittsburgh site 2009-2012)
2011-2013 Gynecologic Center of Excellence. Henry M. Jackson Foundation
2009-2013 Stand up to Cancer (AACR). Bringing epigenetic therapy to the forefront of cancer. (Dream Team Principal with Stephen Baylin and Peter Jones)

2. Seminars and Invited Lectureships

Invited Seminars

1985 Grand Rounds, Washington Veterans Administration Hospital, Washington, DC

1986 Medical Grand Rounds, Johns Hopkins Medical Institutions, Baltimore, MD
Symposium on Breast Cancer, Millville Hospital, Vineland, NJ
Department of Hematology-Oncology, University of Missouri, Kansas City, MO
Kansas City Round Table of Hematology/Oncology, Kansas City, MO
American Association of Osteopathic Internists, Washington, DC
Cincinnati Cancer Conference V, Cincinnati, OH
Advances in Oncology, Cherry Hill, NJ

1987 13th Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders Course,
      Johns Hopkins Medical Institutions, MD
      Breast Cancer Session, Eastern Cooperative Oncology Group, Clearwater, FL

1988 Early Breast Cancer Conference, Memorial Hospital, Colorado Springs, CO
      Grand Rounds, Liberty Medical Center, Baltimore, MD
      Medical Grand Rounds, Johns Hopkins Medical Institutions, Baltimore, MD
      Annual Hematology/Oncology Conference, The Medical Center of Delaware,
      Wilmington, DE
      Department of Medicine Professors Rounds, Johns Hopkins Medical Institutions,
      Baltimore, MD
      Medical Residents Journal Club, Johns Hopkins Medical Institutions, Baltimore, MD
      Plastic Surgery Grand Rounds, Johns Hopkins Medical Institutions, Baltimore, MD

1989 Department of Medicine Bench to Bedside, Johns Hopkins Medical Institutions,
      Baltimore, MD
      Twelfth Annual Symposium on Current Concepts in Medicine and Surgery, Peninsula
      General Hospital, Salisbury, MD
      Topics in Internal Medicine Course, Johns Hopkins Medical Institutions, Baltimore, MD
      15th Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders, Johns
      Hopkins Medical Institutions, Baltimore, MD
      Eleventh Annual Cancer Symposium Selected Topics in Oncology, Raleigh, NC
      Plastic Surgery Grand Rounds, Johns Hopkins Medical Institutions, Baltimore, MD
      St. George's Society, Johns Hopkins Medical Institutions, Baltimore, MD
      Hematology-Oncology Division Seminar, Indiana University School of Medicine,
Indianapolis, IN

1990  Grand Rounds, St. Agnes Hospital, Baltimore, MD
Department of Medicine Bench to Bedside, Johns Hopkins Medical Institutions, Baltimore, MD
American Cancer Society, Maryland Division, Baltimore, MD
Medical Grand Rounds, Johns Hopkins Medical Institutions, Baltimore, MD
16th Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders, Johns Hopkins Medical Institutions, Baltimore, MD
NCI Strategy Meeting on High Dose Chemotherapy in Breast Cancer, National Cancer Institute, Bethesda, MD
Hematology-Oncology Division Seminar, University of Maryland School of Medicine, Baltimore, MD

1991  Hematology-Oncology Conference, Chester Hospital, West Chester, PA
Department of Medicine Professors Rounds, Johns Hopkins Medical Institutions, Baltimore, MD
Symposium on Early Breast Cancer, Montgomery General Hospital, Olney, MD
Hematology-Oncology Division Seminar, Northwestern University School of Medicine, Chicago, IL
Pennsylvania Oncology Society, Gettysburg, PA
Susan G. Komen Foundation Scientific Symposium, University of Texas - Southwestern Medical School. Dallas, TX

1992  Breast Cancer Symposium, Crozer-Chester Hospital, Upland, PA
Hematology-Oncology Grand Rounds, University of Maryland School of Medicine, Baltimore, MD
Department of Medicine Ambulatory Care Rounds, Johns Hopkins Medical Institutes, Baltimore, MD
Staff Conference, Roswell Park Cancer Institute, Buffalo, NY
Tumor Board, Anne Arundel Hospital, Annapolis, MD
18th Annual Symposium on the Diagnosis and Treatment of Neoplastic Disorders, Johns Hopkins Medical Institutions, Baltimore, MD
American Cancer Society, Teaneck, NJ
Australia - New Zealand Breast Cancer Trials Group, Surfers Paradise, Australia
Laboratory of Biologic Chemistry, National Cancer Institute, Bethesda, MD
Lederle Advisory Board, New York City, NY
Gordon Conference on Cancer, Newport, RI
American Society of Clinical Pathologists, Las Vegas, NV
The Cancer Center at Fairfax Hospital, Fairfax, VA
American Fertility Society, New Orleans, LA
Visiting Professor, Department of Medicine, Hahnemann University School of Medicine, Philadelphia, PA

1993  NCI Strategy Meeting on Breast Cancer in Young Women, National Institutes of Health, Bethesda, MD
St. Georges Society, University of Maryland School of Medicine, Baltimore, MD
US-Japanese Joint Scientific Meeting on New Breast Cancer Therapies, Oakland, CA
Isaac Lewin Symposium, Baystate Medical Center, Springfield MA
Discussant, Adjuvant Breast Cancer Session, American Society of Clinical Oncology, Orlando, FL
Educational Session, National Cancer Institute Phase I Meeting, Bethesda, MD
Working Group on the Pulmonary Complications Associated with Breast Cancer Therapy, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD
Shanghai Cancer Institute, Shanghai, Peoples Republic of China
Ethics and Politics in Clinical Trials, Johns Hopkins Medical Institutions, Baltimore, MD
NCI Workshop on Prognostic and Predictive Factors in Breast Cancer, Bethesda, MD

1994
Hematology/Oncology Grand Rounds, Wayne State University School of Medicine, Detroit, MI
Clinical Oncology Program Grand Rounds, National Cancer Institute, Bethesda, MD
NCI Strategy Meeting on High Dose Chemotherapy for Breast Cancer, Bethesda, MD
11th Annual Advances in Cancer Treatment Research, Albert Einstein College of Medicine, New York City, NY
Recent Advances in the Biology of Breast, Colon, and Lung Cancer, American Society of Clinical Oncology, Dallas, TX
Discussant, Plenary Session, American Society of Clinical Oncology, Dallas, TX
Women's Health Seminar Series, Breast Cancer, National Institutes of Health, Bethesda, MD
Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
The State of Breast Cancer 1994: An Interactive Symposium, University of California at San Francisco, San Francisco, CA
Grand Rounds, Washington County Hospital, Hagerstown, MD
Y-ME of the Cumberland Valley, Hagerstown, MD

1995
Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center of West Virginia University, Morgantown, WV
12th Annual International Breast Cancer Conference, Miami, FL
Controversy Session, American Association for Cancer Research, Toronto, Canada
Department of Pharmacology, Mayo Clinic, Rochester, MN
Susan G. Komen Foundation Congressional Breakfast, Washington, DC
Commonwealth of Massachusetts Course on Breast Cancer, Boston, MA
Law and Health Care Program, University of Maryland and Baltimore School of Law, Baltimore, MD
Discussant, Breast Cancer Session, American Society of Clinical Oncology, Los Angeles, CA
Topics in Clinical Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
Gordon Research Conference on Mammary Gland Biology, New London, NH
The Endocrine Society’s 51st Conference on Recent Progress in Hormone Research, Stevenson, WA
Eighteenth Thomas W. Green Memorial Lecture, East Tennessee State University James H. Quillen College of Medicine, Bristol, TN
Fifth International Congress on Hormones and Cancer, Quebec City, Canada
Cancer Medicine, Harvard Medical School, Boston, MA
Medical Oncology Board Review, George Washington University School of Medicine, Washington, DC
The First Annual Kimmel-Slavin Memorial Lecture, George Washington University School of Medicine, Washington, DC
Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
Meet the Professor, American Society of Clinical Oncology Fall Educational Conference, Washington, DC
Grand Rounds, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
Division of Hematology/Oncology, Washington Hospital Center, Washington, DC
18th Annual San Antonio Breast Cancer Symposium, San Antonio, TX
Department of Medicine, St. Joseph Hospital, Baltimore, MD
Dana-Farber Cancer Institute, Boston, MA
22nd Annual Symposium on the Diagnosis and Treatment of Neoplastic Disorders, Johns Hopkins

1996 Department of Embryology, Carnegie Institute of Washington, Baltimore, MD
Mayo Clinic Cancer Center, Rochester, MN
New Approaches to Cancer Therapy, The Johns Hopkins Oncology Center, Baltimore, MD
Topics in Clinical Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
University of Maryland Cancer Center, Baltimore, MD
Discussant, Breast Cancer Session, American Society of Clinical Oncology, Philadelphia, PA
Bowman Gray Comprehensive Cancer Center, Wake Forest University, Winston-Salem, N.C.
American College of Surgeons, San Francisco, CA
Session Chair, Gordon Conference on Cancer Chemotherapy, Oxford, UK
Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
City of Hope National Medical Center, Duarte, CA
Upstate New York Cancer Research and Education Foundation, Syracuse, NY
Wendy and Emery Reeves International Breast Cancer Symposium, University of Texas Southwestern
Medical Center, Dallas, TX 30th Anniversary Symposium, National Institute of Environmental Health Sciences, Research Triangle, NC
Meet the Professor, American Society of Clinical Oncology Fall Educational Conference, Phoenix, AZ
Maryland Cancer Control Symposium, Baltimore, MD
Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC
Department of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD
1997  Breast Cancer Think Tank 7, St. Lucia
        4th Annual Breast Cancer Symposium of the New York Metropolitan Breast Cancer
        Group, New York City, NY
        2nd Annual Multidisciplinary Symposium on Breast Disease, Amelia Island, FL
        University of Colorado Cancer Center, Denver, CO
        Cambridge Symposium, Genetic Approaches to Breast and Prostate Cancer, Lake
        Tahoe, CA
        St. George’s Society, University of Maryland Medical School, Baltimore, MD
        Issues in the Treatment of Breast Cancer, Greater Baltimore Medical Center,
        Baltimore, MD
        University of Chicago Cancer Center, Chicago, IL
        Conjoint Clinic, Johns Hopkins University School of Medicine, Baltimore, MD
        Breast Cancer Tumor Panel, American Society of Clinical Oncology, Denver, CO
        Pittsburgh Cancer Institute, University of Pittsburgh Medical School, Pittsburgh, PA
        International Cancer Alliance, Washington, DC
        Perspectives in Breast Cancer, Emory University, Atlanta, GA
        US Public Health Services Office on Women’s Health Healthy Women 2000,
        Washington, DC
        Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
        American College of Surgeons, Chicago, IL
        Susan G. Komen Foundation Breast Cancer Symposium, Dallas, TX
        Medical Oncology Board Review, George Washington University School of Medicine,
        Washington, DC
        Case Western Reserve University/Ireland Cancer Center, Cleveland, OH
        Holy Cross Hospital, Silver Spring, MD
        Department of Medicine and Cancer Center, University of California at San Francisco,
        San Francisco, CA
        American Society of Clinical Oncology Fall Education Conference, Orlando, FL
        14th Annual American College of Physicians/Army Regional Meeting, Reston, VA
        Fallston Hospital, Fallston, MD

1998  Breast Cancer Think Tank 8, Tobago
        Session Co-Chair, 6th International Conference on Adjuvant Therapy of Primary
        Breast Cancer, St. Gallen, Switzerland
        Controversy Session Chair, American Association for Cancer Research, New Orleans,
        LA
        24th Annual Symposium on Diagnosis and Treatment of Neoplastic Diseases, Johns
        Hopkins Medical Institutions, Baltimore, MD
        Breast Cancer Symposium, Inova Fairfax Hospital, Fairfax, VA
        Discussant, Plenary Session, American Society of Clinical Oncology, Los Angeles, CA
        Department of Pathology, Vanderbilt School of Medicine, Nashville, TN
        Suburban Hospital, Bethesda, MD
        Department of Medicine, Columbia-Presbyterian Medical Center, New York City, NY
        Gordon Conference on Cancer, Newport, RI
Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
2nd Annual Advances in Cancer Therapy, VCU/MCV, Richmond, VA
Kent and Queen Anne’s Hospital, Chestertown, MD
Breast Cancer Awareness Month, The White House Washington, DC
Medical Oncology Board Review, George Washington University School of Medicine, Washington, DC
21st Annual San Antonio Breast Cancer Symposium, San Antonio, TX

1999
Breast Cancer Think Tank 9, St. Thomas, Virgin Islands
Joint Cancer Conference of the Florida Universities, Orlando, FL
Grand Rounds, Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD
Congressionally Directed Medical Research Programs, Frederick, MD
Topics in Internal Medicine, Department of Medicine, Johns Hopkins, Baltimore, MD
American Society of Clinical Oncology, Atlanta, GA
1st Milan Breast Cancer Conference, Milan, Italy
Johns Hopkins Singapore, Singapore
Grand Rounds, Department of Surgery, Northwest Hospital, Baltimore, MD
6th Nottingham International Breast Cancer Conference, Nottingham, England
Seeking Excellence in Breast Cancer Care: Best Practices in Diagnosis and Treatment, Johns Hopkins University School of Medicine, Baltimore, MD
First Annual Lynn Sage Breast Cancer Symposium, Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL
Chemotherapy Symposium, Berlex Oncology Foundation, Leesburg, VA
Cancer Medicine, Harvard Medical School, Boston, MA
41st Annual Meeting of the American Society of Therapeutic and Radiation Oncology, San Antonio, TX
SERMs - Implication for Prevention and Treatment of Cancer, Philadelphia, PA
American Society of Clinical Oncology Fall Education Conference, San Francisco, CA
Genetics Program, University of Missouri, Columbia, MO
22nd Annual San Antonio Breast Cancer Symposium, San Antonio, TX

2000
Sibley Hospital, Washington, DC
Franklin Square Hospital, Baltimore, MD
Molecular Biology of Breast Cancer, Lillehammer, Norway
Keystone Symposium in Advances in Human Breast and Prostate Cancer, Lake Tahoe, NV
NIH Workshop on Selective Estrogen Receptor Modulators (SERMs), Bethesda, MD
Topics in Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
National Breast Cancer Coalition Eighteenth Annual Advocacy Training Conference
2nd Milan Breast Cancer Conference, Milan, Italy
Australia - New Zealand Breast Cancer Trials Group, Queenstown, New Zealand
Suburban Hospital, Bethesda, MD
15th Annual Excalibur Round Table, American Cancer Society, Baltimore, MD
Susan G. Komen Breast Cancer Foundation National Symposium - Reaching for the Cure..... Making a Difference, Washington, DC
WellStar Kennestone Hospital, Marietta, GA
WellStar Cobb Hospital, Marietta, GA
Hematology-Oncology Board Review, George Washington University School of Medicine, Arlington, VA
Berlex Oncology Foundation Clinical Pharmacology of Anticancer Drugs, Leesburg, VA
42nd Annual Meeting of the American Society of Therapeutic and Radiation Oncology, Boston, MA
National Institutes of Health Consensus Development Conference on Adjuvant Therapy of Breast Cancer, Bethesda, MD
Seeking Excellence in Breast Cancer Care, Johns Hopkins University School of Nursing and School of Medicine, Baltimore, MD

2001
Breast Cancer Think Tank 11, Punta Cana, Dominican Republic
Potential Clinical Applications for GnRH Agonists, National Institutes of Health, Bethesda, MD
7th International Conference on Adjuvant Therapy of Primary Breast Cancer, St. Gallen, Switzerland
8th Annual Miami Breast Cancer Conference, Miami, FL
Central Pennsylvania Oncology Group, Harrisburg, PA
Mary E. Humphreys Biology Lecture, Mary Baldwin College, Staunton, VA
Department of Medicine Grand Rounds, Johns Hopkins Bayview, Baltimore, MD
Discussant, American Society of Clinical Oncology, San Francisco, CA
Anne Arundel Medical Center, Annapolis, MD
Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC
Women’s Malignancy Group, MD Anderson Cancer Center, Houston, TX
3rd Milan Breast Conference, Milan, Italy

Gordon Conference on Polyamines, New London, CT
Australian Society for Breast Diseases, Surfers Paradise, Australia (by video conference)
White House/Komen Breast Cancer Summit, Washington, DC
3rd Annual Lynn Sage Breast Cancer Symposium, Northwestern University, Chicago, IL
Hematology-Oncology Board Review, George Washington University School of Medicine, Arlington, VA
Berlex Oncology Foundation Clinical Pharmacology of Anticancer Drugs, Leesburg, VA
Tumor Board, Greater Baltimore Medical Center, Baltimore, MD
Hematology Grand Rounds, Johns Hopkins, Baltimore, MD
William L. McGuire Memorial Lecture, 24th Annual San Antonio Breast Cancer Symposium, San Antonio, TX

2002
Breast Cancer Symposium Think Tank 12, St. Maarten, The Netherlands Antilles
Grand Rounds Department of Medicine, Johns Hopkins University, Baltimore, MD
Current Trends in Breast Cancer, Philadelphia, PA
3rd European Breast Cancer Conference, Barcelona, Spain
The Third North American Symposium on Skeletal Complications of Malignancy, National Institutes of Health, Bethesda, MD
Educational Symposium, American Society for Clinical Oncology, Orlando, FL
4th Milan Breast Cancer Conference, Milan, Italy
Second International Conference on Recent Advances and Future Directions in Endocrine Manipulation of Breast Cancer. Cambridge, MA
Breast Cancer: Current Controversies and New Horizons, Dana Farber Cancer Institute, Boston, MA
Center for Cancer Research Grand Rounds, National Cancer Institute, Bethesda, MD
2nd Annual Karmanos Cancer Institute Breast Cancer Symposium, Detroit, MI
Era of Hope, Department of Defense Breast Cancer Research Program, Orlando, FL
Fox Chase Cancer Center, Philadelphia, PA
IX Congresso Nacional de Oncologia, Lisbon, Portugal

2003  
NCI – Hopkins Workshop on Clinical Translation of Gene Re-expression in Cancer, Baltimore, MD
Breast Cancer Think Tank 13, Aruba
20th Annual Miami Breast Cancer Conference, Miami, FL
8th International Conference on Primary Therapy of Early Breast Cancer, St. Gallen, Switzerland
American Society for Breast Disease, Dallas, TX
Upper Chesapeake Medical Center, Fallston, MD
American Society of Clinical Oncology, Chicago, IL
Gordon Conference on Cancer Chemotherapy, Oxford, UK
National Cancer Institute Workshop on Ductal Lavage, Bethesda, MD
9th Annual Perspectives in Breast Cancer, Boston, MA
Cancer Education Consortium Clinical Pharmacology of Anticancer Agents, Leesburg, VA
Indiana University Cancer Center, Indianapolis, IN
4th Annual Hampton Roads Fall Cancer Conference, Portsmouth, VA
Friends of Cancer Research, Woodrow Wilson International Center for Scholars, Washington, DC
Astrazeneca Breast Cancer Symposium, Waltham, MA

2004  
Breast Cancer Think Tank 14, St Kitts
Translational Conference, Johns Hopkins Oncology Center, Baltimore, MD
Breast Cancer—Bench to Bedside, Loyola University, Chicago, IL
Massachusetts General Hospital, Boston, MA
4th European Breast Cancer Conference, Hamburg, Germany
American Association for Cancer Research, Orlando, FL
The Philip A. Tumulty Topics in Clinical Medicine at Johns Hopkins, Baltimore, MD
Medical Grand Rounds, University of Florida—Shands Medical School, Gainesville, FL
Henry Lemon Memorial Lecture, University of Nebraska—Eppley Cancer Center, Omaha, NE
Discussant, Best of Oncology Symposium, American Society of Clinical Oncology, New Orleans, LA
6th Milan Breast Cancer Symposium, Milan, Italy
Gordon Conference on Molecular Therapeutics of Cancer, New London, NH
7th Annual Mission Conference of the Susan G. Komen Breast Cancer Foundation, Washington, DC
George Washington University Hematology-Oncology Board Review Course, Alexandria, VA
Cancer Education Consortium Clinical Pharmacology of Anticancer Agents, Leesburg, VA
6th Lynn Sage Breast Cancer Symposium of Northwestern University, Chicago, IL
Alta Bates Summit Medical Center, Berkeley, CA
Association of Northern California Oncologists, San Francisco, CA
4th American Association for Cancer Research Prevention Meeting, Seattle, WA
Project LEAD, National Breast Cancer Coalition, Washington, DC
Mayo Clinic Oncology Society, Rochester, MN
Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN
Career Day, Baltimore Polytechnic Institute, Baltimore, MD
2nd Breast Cancer Inter-SPORE Meeting, Chapel Hill, NC
27th Annual San Antonio Breast Cancer Symposium, San Antonio, TX

2005
Greenebaum Cancer Center, University of Maryland Medical School, Baltimore, MD
Breast Cancer Think Tank 15, Curacao
22nd Miami Breast Cancer Symposium, Miami, FL
Lorne Cancer Conference, Phillip Island, Australia
Delaware Oncology Society, Wilmington, DE
New Strategies in Breast Cancer Conference, Philadelphia, PA
Educational Symposium, American Society of Clinical Oncology, Orlando, FL
Highlights of the Day Symposium, American Society of Clinical Oncology, Orlando, FL
National Breast Cancer Coalition Fund Annual Advocacy Conference, Washington, DC
Third International Symposium on the Molecular Biology of Breast Cancer, Molde, Norway
Breast Cancer: Current Controversies and New Horizons, Harvard Medical School, Boston, MA
New England Journal of Medicine Clinical Pathologic Conference, Harvard Medical School, Boston, MA
Hematology-Oncology Board Review, George Washington University Medical Center, Washington, DC
Frances Bull Lecture, University of Michigan, Ann Arbor, MI
University of Minnesota Cancer Center, Minneapolis, MN
50th Anniversary Avon Foundation Symposium, New York City, NY
Cancer Education Consortium Clinical Pharmacology of Anticancer Agents, Leesburg, VA
100 Women Professors Symposium, Johns Hopkins, Baltimore, MD
Working Group on Translational Epigenetics in Cancer, National Cancer Institute, Bethesda, MD
2006  Mayo Clinic, Rochester, MD
Helen Padykula Lecture, Wellesley College, Wellesley, MA
Lynne Abraham Symposium, Susan G. Komen Foundation, New York City, NY
Third Current Concepts in the Multidisciplinary Management of Breast Cancer, Johns Hopkins, Baltimore, DC
Vth Santiago Breast Cancer Symposium, Santiago, Chile
8th Milan Breast Cancer Symposium, Milan, Italy
International Union Against Cancer (UICC) World Cancer Congress, Washington, DC
8th Lynn Sage Breast Cancer Symposium, Chicago, IL
Hematology-Oncology Board Review, George Washington University Medical Center, Washington, DC
Cancer Education Consortium Clinical Pharmacology of Anti-Cancer Agents, Leesburg, VA
44th Meeting of the Japan Society of Clinical Oncology, Tokyo, Japan
National Comprehensive Cancer Network Adjuvant Therapy in Breast Cancer Symposium, Baltimore, MD
Women’s Board, Johns Hopkins Hospital, Baltimore, MD
National Cancer Institute-Ft. Detrick Distinguished Scientist Seminar, Frederick, MD
Science Lecture Series 2006-7 Radcliffe Institute for Advanced Study, Cambridge, MA

2007  Johns Hopkins Workshop on Clinical Targeting of Epigenetic Changes in Cancer Treatment, Phoenix, Arizona
24th Annual Miami Breast Cancer Conference, Miami, FL
6th Annual Mid-Atlantic Oncology Update, St Agnes Hospital, Baltimore, MD
10th International Conference on Primary Therapy of Early Breast Cancer, St. Gallen, Switzerland
Breast Cancer Think Tank 17, Playa del Carmen, Mexico
Annual Advances in Basic Science Symposium, Northwestern University Cancer Center, Chicago, IL
American Society of Clinical Oncology Education Symposium, Chicago, IL
10th Komen Mission Conference, Washington, DC
St Joseph’s Hospital, Baltimore, MD
Australia-New Zealand Breast Cancer Clinical Trials Annual Meeting, Alice Springs, Australia
National Cancer Advisory Board, Bethesda, MD
Hematology-Oncology Board Review, George Washington University Medical Center, Washington, DC
CR-UK Cambridge Research Institute Plenary Lecture, 3rd National Cancer Research Institute Cancer Conference, Birmingham, UK
President’s Cancer Panel, San Diego, CA
Scientific Symposium, Breast Cancer Research Foundation, New York City, NY
Florida Oncology Society, Orlando, FL
Collaborative Summit on Breast Cancer Research, Foundation for the NIH, Lansdowne, VA
American Association of Cancer Research Prevention Symposium, Philadelphia, PA

2008  
7th Rosalind E. Franklin Award for Women in Science, National Cancer Institute, Bethesda, MD  
Breast Cancer Think Tank 18, Waikalaoa, HI  
Cancer Institute of New Jersey, New Brunswick, NJ  
5th Early Detection Research Network Scientific Workshop, National Cancer Institute, Bethesda, MD  
Vanderbilt-Ingram Comprehensive Cancer Center, Nashville, TN  
11th American Association for Cancer Research—Women in Cancer Research Charlotte Friend Award, San Diego, CA  
14th Annual Educational Symposium, Susan G. Komen for the Cure Maryland, Baltimore, MD  
4th Current Concepts in the Multidisciplinary Management of Breast Cancer, Johns Hopkins University, Baltimore, MD  
Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA  
Best of ASCO, Boston, MA  
Annual Meeting of the American Association for Clinical Chemistry, Washington, DC  
Seventh International Congress on the Future of Breast Cancer, Kauai, HI  
Nuclear Hormone Receptors, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY  
Hematology-Oncology Board Review, George Washington University, Washington, DC  
Fourth Annual Oncology Congress, San Francisco, CA  
Oberstar Lecture, George Washington University, Washington, DC

2009  
Breast Cancer Think Tank 19, Costa Rica  
Bernard Fisher Lecture, University of Pittsburgh, Pittsburgh PA  
11th International Congress Oncology Conference on Primary Therapy of Early Breast Cancer, Saint Gallen, Switzerland  
Grand Rounds, MD Anderson Cancer Center – Houston TX  
Women Leading the Way, MD Anderson Cancer Center, Houston, TX  
Jean Sindab Lecture, Emory Winship Cancer Institute, Atlanta, GA  
Oncology Grand Rounds, Ohio State University, Columbus, OH  
16th Annual Pennsylvania Bar Association Women in the Profession, Pittsburgh, PA  
American Society of Clinical Oncology Educational Symposium, Orlando, FL  
24th Annual Aspen Cancer Conference, Aspen, CO  
Harvard Breast Cancer Conference, Boston, MA  
Medical Grand Rounds, UPMC Shadyside Hospital, Pittsburgh, PA  
University of Pittsburgh Postdoctoral Association Data and Dine Lecture, Pittsburgh, PA  
Women's Studies and the Provost's Advisory Committee on Women's Concerns New Faculty Lecture, University of Pittsburgh, Pittsburgh, PA  
Pancreasfest 2009, University of Pittsburgh, Pittsburgh, PA  
AACR Advances in Breast Cancer Research, San Diego, CA  
Cincinnati Cancer Symposium, Jensen Symposium on Nuclear Receptors, Cincinnati, Ohio  
Translating Scientific Advances into Clinical Care Cancer, Lineberger Comprehensive
Cancer Center, Chapel Hill, NC
New Options in Breast Cancer Treatment, UPMC Cancer Centers, Johnstown, PA

2010  
University of Pittsburgh Winter Academy, Naples, FL  
Achievement Rewards for College Scientists, Pittsburgh, PA  
Medical Grand Rounds, UPMC Montefiore University Hospital, Pittsburgh, PA  
Katz Lecture, Magee Womens Hospital of Pittsburgh, Pittsburgh, PA  
NYU Cancer Institute Seminar Series, New York, NY  
The Regional Cancer Center, Erie, PA  
Lesses Visiting Professor, Medical Grand Rounds, Beth Israel Deaconess Medical Center, Boston, MA  
Hematology-Oncology Grand Rounds, Beth Israel Deaconess Medical Center, Boston, MA  
University of Maryland Marlene and Stewart Greenebaum Cancer Center, Hormone Responsive Cancer Program Retreat, Baltimore, MD  
Lois O’Grady Breast Cancer Lecture, University of California Davis Cancer Center, Sacramento, CA  
11th Annual Advances in Oncology, University of California Davis Cancer Center, Sacramento, CA  
American Society of Clinical Oncology Breast Cancer Symposium - Gianni Bonadonna Award, Washington, DC  
Advances in Oncology, Keynote speaker, UPMC Beacon Hospital, Ireland  
Oncology Grand Rounds, Thomas Jefferson University Kimmel Cancer Center, Philadelphia, PA

2011  
Dept of Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA  
Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA  
Breast Cancer Program Retreat, UCSF Cancer Center, San Francisco, CA  
Lineberger Cancer Center, UNC Chapel Hill, Chapel Hill, NC  
XI Michelangelo Foundation Seminar, Milan Italy  
Georgetown University, Undergraduate Research Conference Keynote Speaker, Washington, DC  
City of Hope Cancer Center Grand Rounds, Duarte, CA  
Cleveland Clinic Grand Rounds, Taussig Cancer Center, Cleveland, Ohio  
McArdle Laboratory Seminar, University of Wisconsin, Madison, WI  
13th Milan Breast Cancer Conference, Milan, Italy  
Annual Meeting, American Society of Clinical Oncology, Chicago, IL  
International Cancer Conference, Trinity Medical School, Dublin, Ireland  
Fifth Annual Ri.MED Scientific Symposium, Palermo, Italy  
Medical Oncology Board Review, George Washington University, Washington, DC  
Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA  
Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pgh, PA  
San Antonio Breast Cancer Symposium, San Antonio, TX

2012  
Breast Cancer Think Tank 22, Mexico
Siteman Cancer Center at Washington University, Saint Louis, MO
Miami Breast Cancer Conference, Miami, FL
University of Pittsburgh Department of Pathology, Pittsburgh, PA
American Society of Preventive Oncology, Washington, DC
Tsinghua University, Beijing, China
University of Pittsburgh, Chancellors Inaugural Lecture - Hillman Professor of Oncology, Pittsburgh, PA
University of Chicago Cancer Biology Seminar Series, Chicago, IL
Johns Hopkins University School of Medicine, Baltimore, MD
American Society of Clinical Oncology, Chicago, IL
34th Annual Scientific Meeting, Australia-New Zealand Breast Cancer Trials Group, Hobart, Australia
Medical Oncology Board Review, George Washington University, Washington, DC
University of Texas Southwestern, Pamela Hearn Isom Lecture, Medicine Grand Rounds, Dallas Texas
Potamkin Lecture, PA Breast Cancer Coalition Conference, Harrisburg, PA
Northwestern University Feinberg School of Medicine's 16th Annual Department of Pathology Joseph C. Calandra Lecture, Chicago, IL
The Shanghai Breast Cancer Symposium, Shanghai, China

2013
Bay City Capital Scientific Advisory Board Meeting
13th International Congress Oncology Conference on Primary Therapy of Early Breast Cancer, St. Gallen, Switzerland
Annual Meeting, American Association of Cancer Research, Washington, DC
Case Western Reserve Comprehensive Cancer Center, Cleveland, Ohio
Medical Oncology Board Review, George Washington University, Washington, DC
The Hong Kong University of Science and Technology, Tetralateral Symposium, Hong Kong
PA Cancer Planning Summit, Pittsburgh, PA
Breast Cancer Symposium, San Francisco, CA
Cancer Caucus, House of Representatives, Harrisburg, PA
Science 2013, Pittsburgh, PA
Global Breast Cancer Conference, Seoul, South Korea

2014
Annual Meeting, American Association for Cancer Research, San Diego, CA
American Society of Clinical Oncology, Chicago, IL
German Cancer Research Center (DKFZ), Heidelberg, Germany
Medical Oncology Board Review, George Washington University, Washington, DC
National Cancer Advisory Board, Bethesda, MD
International Oncology Symposium, Astana, Kazakhstan
University of Chicago Simon M. Shubitz Lecture, Chicago, IL
Congressional Briefing, Alliance for Health Reform, Washington, DC
San Antonio Breast Cancer Conference, San Antonio, TX

2015
14th St. Gallen International Breast Cancer Conference, Primary Therapy of Early Breast Cancer, Vienna, Austria
Pediatric Hematology/Oncology Pediatric Hematology/Oncology BMT & CT Conference, Childrens Hospital of Pittsburgh, Pittsburgh, PA
University of Pittsburgh, Winter Academy, Palm Beach, Florida
The Wistar Institute, Distinguished Lecture, Philadelphia, PA
Annual Meeting, American Association for Cancer Research, Philadelphia, PA
Inaugural Lecture as Distinguished Professor of Medicine, University of Pittsburgh, Pittsburgh PA
Stephen D Williams, MD Lectureship, Indiana University Simon Cancer Center, Indianapolis, IN
Medical Oncology Board Review, George Washington University, Washington, DC
ASCO 2015 Breast Cancer Symposium, San Francisco, CA
Taipei Medical University, Taipei, Taiwan
Eighth Annual Robert B. Dickson Memorial Lectureship, Georgetown University Lombardi Cancer Center, Washington DC
First Gabriel Hortobagyi Lecture, MD Anderson Cancer Center, Houston, TX
Lynn Sage Distinguished Lecture, Robert H. Curie Comprehensive Cancer Center of Northwestern University, Chicago, IL

2016 University of Pittsburgh, Winter Academy, Palm Beach, Florida
American Association for Cancer Research (AACR), 2016 Annual Meeting, New Orleans, LA
American Society of Clinical Oncology (ASCO), 2016 Annual Meeting, Chicago, IL
Maryland Breast Cancer Consortium, Baltimore, MD
AACR High Tech Strategic Business Meeting, Sunnyvale, CA
Medical Oncology Board Review, George Washington University, Washington, DC
Great Lakes Breast Cancer Symposium, University of Pittsburgh, Pittsburgh, PA
Seattle Cancer Care Alliance, Anchorage, Alaska
2016 Cooper Lecture, University of Pittsburgh, Pittsburgh, PA

3. Other Research Related Activities

PATENTS GRANTED:


EDITORIAL BOARDS:

1995-2005 Cancer Research
1995-2009 The Breast Journal
1996-2011 The Breast
1997-2005 American Journal of Medicine
1999-2005 Clinical Cancer Research

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2007-2014  Hem/Onc Today
2008-present  Oncology
2008-present  Cancer Prevention Research
2012-present  Journal of the National Cancer Institute
2012-present  Breast Cancer Research and Treatment

**STUDY SECTION MEMBERSHIPS:**

1988  Member, Ad Hoc Technical Review Section, National Cancer Institute, Bethesda, MD
1990, 1991, 1992  Ad Hoc Member, Reproductive Endocrinology Study Section, National Institutes of Health, Bethesda, MD
1992-1993  Member, Awards Committee, Susan G. Komen Foundation, Dallas, TX
1993-1997  Member, Reproductive Endocrinology Study Section, National Institutes of Health, Bethesda, MD
1994  Member, Walt Disney – American Cancer Society Breast Cancer Professorship Selection Committee, Atlanta, GA
1997-1998  Co-Chair, Progress Review Group for Breast Cancer Research, National Cancer Institute, Bethesda, MD
1996-1998  Chair, Pre-clinical and Clinical Studies Study Section, California Breast Cancer Research Program, San Francisco, CA
1999-present  Medical Advisory Board, Breast Cancer Research Foundation, New York City, NY
2001  Member, Breast and Prostate SPORE Review Group, National Cancer Institute
2002  Co-chair, Breast Cancer SPORE Review Group, National Cancer Institute
2003-2008  Vice-Chair, National Cancer Institute Breast Cancer Intergroup Correlative Science Committee
2002-2005  Member, Charles Kettering Prize Selection Committee, General Motors Cancer Research Foundation, Chair, 2005
2003  Chair, Innovator Award Review Committee, Department of Defense Breast Cancer Program
2005  Member, Lung and Bladder Cancer SPORE Review Group, National Cancer Institute
2005-2006  Ad hoc member, Kimmel Scholars Award Committee
2006-present  Member, Kimmel Scholars Award Committee
2006-2010  Member, Subcommittee A – Cancer Centers, National Cancer Institute
2008  Co-chair, Lung Cancer and Lymphoma SPORE Review Group, National Cancer Institute
2008-present  Member, Scientific Advisory Board, V Foundation for Cancer Research
2008  Chair, Therapeutic Targets I Review Committee, Susan G Komen for the Cure
2012-present  Member, Damon Runyon Cancer Research Foundation Clinical Investigator Award Committee
2012-2013  Chair of the Cancer Program Review, Helmholtz Senate Commission, Helmholtz Association of German Research Centers, Berlin, Germany
2014  Member, Scientific Advisory Committee, Breakthrough Breast Cancer, London, UK
2014- Chair, CTAC SPORE Program Evaluation Working Group, NCI
2015Chair, Stand Up To Cancer Canada-Canadian Breast Cancer Foundation Breast Cancer Dream Review Team Committee

EXTRA-MURAL GRANT REVIEWING:

Ad hoc grant reviewer for: National Institutes of Health, American Cancer Society, Veterans Administration, Manitoba (Canada) Health Council, Health Research Council of New Zealand, National Cancer Institute - Canada, Medical Research Council - Canada, Department of Defense Breast Cancer Program, many others

ADVISORY BOARD MEMBERSHIPS:

2000-present Member, External Advisory Board, Vanderbilt-Ingram Cancer Center, Nashville, TN
2001-2006 Member, External Advisory Board, Fox Chase Cancer Center, Philadelphia, PA
2001-2011 Member, External Advisory Board, Bay Area UCSF Breast Cancer SPORE, San Francisco, SF
2003-present Member, External Advisory Board, Karmanos Cancer Center, Detroit, MI
2003-2008 Member, External Advisory Board, Indiana University Cancer Center, Indianapolis, IN
2005-2016 Member, External Advisory Board, University of Maryland Cancer Center, Baltimore, MD
2008-present Member, Board of Scientific Consultants, Memorial Sloan Kettering Cancer Center, New York City, NY
2008-present Member, External Advisory Board, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, Chair 2014-
2009-present Member, External Advisory Board, MD Anderson Cancer Center, Houston, TX Chair 2014-
2010-present Member, External Advisory Board, University of Michigan Comprehensive Cancer Center
2010-present Member, External Advisory Board, Washington University Siteman Cancer Center, St. Louis, MO
2010-present Member, External Advisory Board Breast Cancer SPORE, Mayo Clinic, Rochester, MN
2010-present Member, External Advisory Board, Institut National du Cancer, Paris, France
2011-present Member, External Advisory Board, Fred Hutchinson Cancer Research Center University of Washington Cancer Consortium, Seattle, Washington
2011-present Member, Scientific Advisory Board, CTSA, Case Western Reserve University School of Medicine, Cleveland, Ohio
2012-present Member, Scientific Advisory Board, Cologne Center for Integrated Oncology, Cologne, Germany
2013-present Member, External Advisory Board, Baylor College of Medicine Breast Cancer SPORE
2014-present Member, External Advisory Board, University of Chicago Comprehensive Cancer Center, Chicago, IL
2014-2015 Member, Scientific Advisory Board, A. Alfred Taubman Medical Research Institute, University of Michigan, Ann Arbor, MI

LIST OF CURRENT RESEARCH INTERESTS

Cancer biology and treatment, especially breast cancer

SERVICE

1. UNIVERSITY AND MEDICAL SCHOOL:
   Committees - Johns Hopkins
   1987 Co-Director, Oncology Multidisciplinary Conference
   1987-1997 Member, Oncology Fellowship Selection Committee
   1993-1995 Department Representative, Medical School Council
   1999-2003 Departmental Appointments and Promotions Committee
   2000-2005 Member, School of Medicine Professorial Promotion Committee
   2001-2009 Member, MD-PhD Admissions Committee
   2003-2005 Member, Search Committee for Director of Biophysics and Biophysical Chemistry

   Committees – University of Pittsburgh, University of Pittsburgh Physicians (UPP), UPMC
   2009-present Member, Chair Management Committee
   2009-present Member, UPP Clinical Chairs Committee
   2009-present Member, Breast Cancer Steering Committee
   2009-present Member, Clinical Research Oversight Committee
   2009-2012 Member, ReSet Steering Committee
   2009-present Member, Adolescent and Young Adult Cancer Committee
   2010-2011 Member, Search Committee for Department of Medicine Chairman
   2011-2012 Member, Search Committee for the Institute of Personalized Medicine
   2012-present Member, UPMC Presbyterian Shadyside Hillman Cancer Committee
   2012-present Member, School of Medicine Financial Oversight Committee
   2014-present Member, Internal Advisory Board Gynecologic SPORE
   2015-present Member, Internal Advisory Board for the Center for Causal Discovery
   2015-present Member, Internal Advisory Board of the Center for Medical Counter Measures Against Radiation (CMCR)

2. EXTERNAL ORGANIZATIONS:
   1993-1995 Member, Medical Advisory Committee, Maryland Cancer Consortium
   1994-1996 Executive Committee, American Cancer Society – Maryland Division Chair, Research Committee
   1995-1998 Member, Medical Knowledge Self Assessment Program 11-Oncology, American College of Physicians
1999-2000 Planning Committee, National Institutes of Health Consensus Development Conference on Adjuvant Therapy for Breast Cancer, Bethesda, MD
1998-2001 Data Monitoring Committee, Southwest Oncology Group
1999-2006 Data Monitoring Committee, RUTH Trial, Lilly
2000-2006 Data Monitoring Committee, Breast Cancer International Research Group (BCIRG)
2006-2011 Member, Data and Safety Monitoring Committee, TEACH Trial, Glaxo Smith Kline
2009-2012 NMR Center Advisory Committee, Carnegie Mellon University
2010-2016 Co-Chair, Breast Cancer Steering Committee, National Cancer Institute
2011-present Member, Clinical Trials and Translational Research Advisory Committee (CTAC), National Cancer Institute, Chair 2015-present
2013-present Board of Trustees, Phipps Conservatory, Pittsburgh, PA
2015- Member, Search Committee for the Scientific Director (SD), Center for Cancer Research (CCR), National Cancer Institute
2015- Member, Breast Cancer Now’s Science Strategy Committee
We have previously demonstrated that crosstalk between lysine-specific demethylase 1 (LSD1) and histone deacetylases (HDACs) facilitates breast cancer proliferation. However, the underlying mechanisms are largely unknown. Here, we report that expression of HDAC5 and LSD1 proteins were positively correlated in human breast cancer cell lines and tissue specimens of primary breast tumors. Protein expression of HDAC5 and LSD1 was significantly increased in primary breast cancer specimens in comparison with matched-normal adjacent tissues. Using HDAC5 deletion mutants and co-immunoprecipitation studies, we showed that HDAC5 physically interacted with the LSD1 complex through its domain containing nuclear localization sequence and phosphorylation sites. Although the in vitro acetylation assays revealed that HDAC5 decreased LSD1 protein acetylation, small interfering RNA (siRNA)-mediated HDAC5 knockdown did not alter the acetylation level of LSD1 in MDA-MB-231 cells. Overexpression of HDAC5 stabilized LSD1 protein and decreased the nuclear level of H3K4me1/me2 in MDA-MB-231 cells, whereas loss of HDAC5 by siRNA diminished LSD1 protein stability and demethylation activity. We further demonstrated that HDAC5 promoted the protein stability of USP28, a bona fide deubiquitinase of LSD1. Overexpression of USP28 largely reversed HDAC5-KO-induced LSD1 protein degradation, suggesting a role of HDAC5 as a positive regulator of LSD1 through upregulation of USP28 protein. Depletion of HDAC5 by shRNA hindered cellular proliferation, induced G1 cell cycle arrest, and attenuated migration and colony formation of breast cancer cells. A rescue study showed that increased growth of MDA-MB-231 cells by HDAC5 overexpression was reversed by concurrent LSD1 depletion, indicating that tumor-promoting activity of HDAC5 is an LSD1 dependent function. Moreover, overexpression of HDAC5 accelerated cellular proliferation and promoted acridine mutagen ICR191-induced transformation of MCF10A cells. Taken together, these results suggest that HDAC5 is critical in regulating LSD1 protein stability through post-translational modification, and the HDAC5–LSD1 axis has an important role in promoting breast cancer development and progression.
mechanisms underlying the functional link between LSD1 and HDAC5 in chromatin remodeling and demonstrated that these two important chromatin modifiers closely cooperate to mediate proliferation, cell cycle and metastasis of breast cancer cells.

RESULTS

HDAC5 and LSD1 proteins are coordinately expressed in human breast cancer

To study the potential association of HDAC5 and LSD1 in breast cancer, we first examined mRNA levels of HDAC5 and LSD1 in human immortalized normal mammary epithelial MCF10A cells, fully malignant MCF10A-CA1a cells transformed from MCF10A cells with transfection of HRAS,15 and several human breast cancer cell lines. Quantitative PCR (qPCR) studies showed that there was no clear association of mRNA expression between HDAC5 and LSD1 in breast cancer cell lines (Figure 1a). The Oncomine-TCGA database showed moderate change of the mRNA level of LSD1 and HDAC5 in IBC (Supplementary Figures 1a and b). mRNA levels of both HDAC5 and LSD1 were altered in ~6% of breast cancer patients (www.cbioportal.org) without an apparent association with specific subtypes (Supplementary Figures 1c and d). However, protein expression of both HDAC5 and LSD1 was significantly elevated in malignant breast cell lines compared with MCF10A (Figure 1b), and protein levels of HDAC5 and LSD1 were positively correlated (Figure 1c). The correlation of HDAC5 and LSD1 protein expression was further validated in 50 primary breast cancers using immunohistochemical staining with validated antibodies (Supplementary Figures 2a and b). The χ² analysis showed a statistically significant correlation between HDAC5 and LSD1 protein expression in these tumors (Figure 1d). Furthermore, the immunohistochemistry (IHC) analysis showed that breast cancer tissues (n = 18) expressed significantly higher level of HDAC5 and LSD1 than matched-normal adjacent tissues (n = 18) (Figure 1e). The mean H-score for HDAC5 staining in stage 3 breast tumors (n = 25) was statistically significantly higher than stage 2 counterparts (n = 25). The mean H-score of LSD1 staining for stage 3 tumors was also higher than that of stage 2 tumors with a P-value of 0.07 (Figure 1f). These results were further validated with independent manual H-score evaluations by two breast cancer pathologists with moderate interobserver concordance (Supplementary Figures 3a and b). Taken together, these findings suggest that HDAC5 and LSD1 proteins are coordinately overexpressed in breast cancer cell lines and tissue specimens.

Physical interaction of LSD1 and HDAC5 in breast cancer cells

To address whether LSD1 and HDAC5 physically interact, a co-immunoprecipitation study was carried out in MDA-MB-231 and MCF10A-CA1a cells transiently transfected with pcDNA3.1 or pcDNA3.1-FLAG-HDAC5 plasmids. After immunoprecipitation (IP) with LSD1 antibody, we found that both endogenous and exogenous HDAC5 proteins were co-immunoprecipitated with LSD1 protein (Figure 2a). The interaction between native LSD1 and HDAC5 was further validated in additional breast cancer cell lines (Figure 2b). A similar result was obtained in the reciprocal immunoprecipitation using anti-FLAG antibody to confirm that HDAC5 was co-immunoprecipitated with FLAG-HDAC5 (Figure 2c). To precisely map the HDAC5 domain(s) responsible for interaction with LSD1, we expressed a series of HDAC5 deletion mutants engineered in pcDNA3.1-FLAG plasmids in MDA-MB-231 cells (Figure 2d). Immunoprecipitation assays of cells transfected with full-length HDAC5 complimentary DNA (cDNA) confirmed the HDAC5–LSD1 interaction and deletion of an N-terminal myocyte enhancer factor-2 (MEF2) binding domain (HDAC5–Δ1) alone had no impact on HDAC5-LSD1 interaction. However, removal of both the MEF2 domain and nuclear localization sequence (NLS) (HDAC5–Δ2) completely abolished HDAC5–LSD1 interaction. Further deletion of an N-terminal HDAC and nuclear export sequence (HDAC5–Δ3) and MEF2 domain (HDAC5–Δ4) did not adversely alter LSD1 binding with HDAC5 fragments (Figure 2e). Immunofluorescence studies showed nuclear localization of full-length HDAC5, HDAC5–Δ1, HDAC5–Δ3 and HDAC5–Δ4. Depletion of the NLS-containing domain (HDAC5–Δ2) completely blocked HDAC5 nuclear translocation (Figure 2f). In vitro pull-down assays by using His-tag recombinant LSD1 protein incubating with HDAC5 full-length or deletion mutants validated that HDAC5 domain containing NLS element is essential for interaction with LSD1 (Supplementary Figure 4).

HDAC5 promotes LSD1 protein stability and activity

Next, we examined whether the mRNA or protein levels of HDAC5 and LSD1 were affected by their interaction with each other. Overexpression of HDAC5 in MDA-MB-231 cells failed to alter LSD1 mRNA expression, but led to a significant increase of LSD1 protein expression (Figures 3a and b). HDAC5 knockdown by siRNA attenuated LSD1 protein expression without affecting its mRNA level (Figures 3c and d). The effect of LSD1 on HDAC5 expression was subsequently assessed using our previously established MDA-MB-231-LSD1-KD cells.10 Depletion of LSD1 exerted no effect on HDAC5 mRNA or protein levels (Figures 3e and f). Simultaneous overexpression of pcDNA3.1-HDAC5 with HDAC5 siRNA significantly reversed the decrease of LSD1 (Supplementary Figure 5a). These results suggest that HDAC5 functions as an upstream regulator that governs LSD1 protein stability via post-translational regulation. Quantitative immunoblots showed that levels of H3K4me1/2 and AcH3K9, the substrates for LSD1 and HDAC5, respectively, were downregulated by HDAC5 overexpression, whereas loss of HDAC5 exerted the opposite effect (Figure 3g; Supplementary Figure 5b), suggesting a critical role of HDAC5 in governing chromatin modifying activity of LSD1. The cycloheximide chase assay showed that overexpression of HDAC5 significantly extended LSD1 protein half-life, whereas depletion of HDAC5 by siRNA decreased LSD1 protein half-life in MDA-MB-231 cells (Figures 3h and i; Supplementary Figure 5c). To determine whether other recognized LSD1 cofactors or HDACs exert similar effects on LSD1 protein stability, MDA-MB-231 cells were treated with siRNA against several LSD1 complex cofactors (CoREST, HDAC1 and HDAC2) or other class II HDAC isozymes (HDAC 4, 6, 7, 9, 10), respectively. Transfection with siRNA probes effectively knocked down mRNA expression of target genes without affecting LSD1 protein level (Figure 3j; Supplementary Figure 6a). To confirm the qPCR results, quantitative immunoblotting (IB) was performed and showed depletion of...
CoREST led to insignificant change of LSD1 protein stability (Supplementary Figure 6b and 6c). Together, these results strengthen the conclusion that HDAC5 functions as a positive regulator of LSD1 protein in breast cancer cells.

HDAC5 regulates LSD1 protein stability through modulation of the LSD1-associated ubiquitination system.

Protein ubiquitination assays indicated that HDAC5 overexpression significantly attenuated LSD1 polyubiquitination (Figure 4a),
whereas depletion of HDAC5 by siRNA facilitated LSD1 polyubiquitination (Supplementary Figure 7a). Recently, Jade-2 and USP28 were identified as specific E3 ubiquitin ligase and deubiquitinase for LSD1, respectively.20,21 Our study showing that increase of LSD1 protein expression by Jade-2 siRNA and decrease of LSD1 protein expression by USP28 siRNA in MDA-MB-231 cells confirmed the roles of Jade-2/USP28 as LSD1 ubiquitin ligase/deubiquitinase in breast cancer cell lines. IgG was used as negative control. (c) MDA-MB-231 cells were transfected with control vector pcDNA3.1 or pcDNA3.1-HDAC5 plasmids, and IP was performed with anti-LSD1 antibody followed by immunoblotting (IB) with anti-LSD1, anti-FLAG or anti-HDAC5 antibodies, respectively. (d) Whole-cell lysates were immunoprecipitated with anti-LSD1 antibody followed by IB with anti-HDAC5 and LSD1 antibodies in indicated breast cancer cell lines. IgG was used as negative control. (e) MDA-MB-231 cells were transfected with control vector pcDNA3.1 or pcDNA3.1-HDAC5-FLAG plasmids, and IP was performed with anti-FLAG followed by IB with anti-LSD1 and anti-FLAG antibodies, respectively. (f) Schematic representation of full-length and deletion mutants of HDAC5-FLAG constructs. (g) FLAG-tagged full-length or deletion mutants of HDAC5 were expressed in MDA-MB-231 cells. Extracts were immunoprecipitated with anti-FLAG antibody, and bound LSD1 was examined by IB using anti-LSD1 antibody. IB with anti-FLAG was used to detect the levels of FLAG-tagged HDAC5 full-length or deletion mutants in IP and input (10%) samples. (h) MDA-MB-231 cells were transfected with plasmids expressing FLAG-tagged full-length or deletion mutants of HDAC5 proteins. Immunofluorescence study was performed using anti-FLAG antibody. 4,6-Diamidino-2-phenylindole was used as a control for nuclear staining. All the experiments were performed three times with similar results.

Figure 2. HDAC5 and LSD1 physically interact in breast cancer cells. (a) MDA-MB-231 or MCF10A-CA1a cells were transfected with control vector pcDNA3.1 or pcDNA3.1-HDAC5 plasmids. IP was performed with anti-LSD1 antibody followed by immunoblotting (IB) with anti-LSD1, anti-FLAG or anti-HDAC5 antibodies, respectively. (b) Whole-cell lysates were immunoprecipitated with anti-LSD1 antibody followed by IB with anti-HDAC5 and LSD1 antibodies in indicated breast cancer cell lines. IgG was used as negative control. (c) MDA-MB-231 cells were transfected with control vector pcDNA3.1 or pcDNA3.1-HDAC5-FLAG plasmids, and IP was performed with anti-FLAG followed by IB with anti-LSD1 and anti-FLAG antibodies, respectively. (d) Schematic representation of full-length and deletion mutants of HDAC5-FLAG constructs. (e) FLAG-tagged full-length or deletion mutants of HDAC5 were expressed in MDA-MB-231 cells. Extracts were immunoprecipitated with anti-FLAG antibody, and bound LSD1 was examined by IB using anti-LSD1 antibody. IB with anti-FLAG was used to detect the levels of FLAG-tagged HDAC5 full-length or deletion mutants in IP and input (10%) samples. (f) MDA-MB-231 cells were transfected with plasmids expressing FLAG-tagged full-length or deletion mutants of HDAC5 proteins. Immunofluorescence study was performed using anti-FLAG antibody. 4,6-Diamidino-2-phenylindole was used as a control for nuclear staining. All the experiments were performed three times with similar results.
completely blocked the destabilization of LSD1 by HDAC5 depletion (Figure 4f; Supplementary Figure 7d). In an additional rescue experiment, overexpression of HDAC5 failed to promote LSD1 protein expression when cells were simultaneously treated with USP28 by siRNA (Supplementary Figure 7e). All these data support the notion that HDAC5 stabilizes LSD1 protein by enhancing protein expression of its deubiquitinase.

To examine whether interaction of HDAC5 with the LSD1/USP28 complex deacetylates LSD1 or USP28, in vitro protein acetylation assays was first carried out by incubating GST-tagged recombinant HDAC5–LSD1 axis in breast cancer

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Figure 4. HDAC5 regulates LSD1 by altering USP28 stability. (a) MDA-MB-231 cells transfected with pcDNA3.1-FLAG, pcDNA3.1-FLAG-HDAC5 or pcDNA3-HA-ubiquitin plasmids were treated with or without proteasome inhibitor 10 μM MG132 for 10 h followed by IP using LSD1 antibody and immunoblots with anti-HA, LSD1 or HDAC5 antibodies. (b) Effect of siRNA of Jade-2, USP28 and HDAC5 on LSD1 protein expression in MDA-MB-231 cells. Results represent the mean of three independent experiments ± s.d. ***P < 0.001, Student’s t-test. (c) MDA-MB-231 cells were transfected with scramble siRNA, HDAC5 siRNA, control vector pcDNA3.1 or pcDNA3.1-HDAC5 plasmids for 48 h. mRNA expression of Jade-2 and USP28 was measured by qPCR. β-actin was used as an internal control. (d) MDA-MB-231 or MCF10A-CA1a cells were simultaneously transfected with pcDNA3.1-FLAG-Jade-2 and HDAC5 siRNA for 48 h and subjected to immunoblots with anti-HDAC5 or Jade-2 antibodies. β-actin was used as loading control to normalize target protein levels. (e) After MDA-MB-231 or MCF10A-CA1a cells were transfected with control vector pcDNA3.1 or pcDNA3.1-HDAC5 plasmids for 48 h, IB was performed for expression of HDAC5 and USP28. (f) MDA-MB-231 or MCF10A-CA1a cells were transfected with scramble or HDAC5 siRNA alone, or in combination with pDZ-USP28 for 48 h. Whole-cell lysates were analyzed for protein levels of HDAC5, USP28 and LSD1. β-actin was used as loading control to normalize target protein levels. The experiments were performed three times with similar results.
Figure 5. Effect of HDAC5 on protein acetylation of LSD1/USP28 and transcription of LSD1 target genes. (a) The immunoprecipitates of FLAG using FLAG-M2 agarose from MDA-MB-231 cells overexpressing FLAG-tagged USP28 or FLAG-tagged LSD1 were used as substrates for protein deacetylation assay. IgG was used as negative control. Active or heat inactivated recombinant human GST-tagged HDAC5 protein were mixed with immunoprecipitates and incubated at 37 °C for 6 h as described in ‘Materials and Methods’. The reactions were then subjected to immunoblots with anti-acetyl lysine antibody. FLAG-tagged USP28 or LSD1 proteins were probed with anti-FLAG antibody. HDAC5-GST protein was probed with anti-HDAC5 antibody. (b) Histograms represent the means of levels of acetyl-LSD1, acetyl-USP28 and acetyl-histone determined by quantitative IB using infrared IB detection and analysis. (c) MDA-MB-231 cell transfected with scramble or HDAC5 siRNAs for 48 h. LSD1 or IgG antibodies were added to cell lysate. IP was performed with anti-LSD1 antibody followed by IB with anti-acetyl lysine and anti-LSD1 antibodies, respectively. Effect of HDAC5 siRNA on AcetylH3K9 protein expression in MDA-MB-231 cells was examined by IB with anti-acetyl-H3K9 antibody. (d) Histograms represent the means of relative levels of acetyl-LSD1 determined by quantitative IB using infrared IB detection and analysis. (e) mRNA expression of indicated genes in MDA-MB-231 cells transfected with scramble siRNA or HDAC5 siRNA. Data are means ± s.d. of three independent experiments. (f) Quantitative chromatin immunoprecipitation (ChIP) analysis was used to determine the occupancy by acetyl-H3K9, H3K4me2, LSD1 and HDAC5 at promoters of p21 or CLDN7 in MDA-MB-231 cells transfected with scramble or HDAC5 siRNA. *P < 0.05, **P < 0.01, ***P < 0.001, Student’s t-test.
HDAC5 protein with cellular pull-down of LSD1-FLAG or USP28-FLAG by IP, and immunoprecipitates of IgG was incubated with recombinant HDAC5 protein as negative control of assays (Figure 5a). Bulk histone was used as control substrate (Supplementary Figure 8). Quantitative immunoblots using antibody against pan-acetylated lysine showed that HDAC5 reduced acetylation level of LSD1 without altering the acetylation status of USP28 (Figures 5a and b). Next, the in vivo effect of HDAC5 depletion on LSD1 acetylation was investigated in MDA-MB-231 cells transfected with scramble or HDAC5 siRNAs. After immunoprecipitation with LSD1 antibody or IgG (negative control), IB was performed and the results showed that expression levels of both...
total LSD1 protein and acetylated LSD1 protein were decreased by HDAC5 depletion (Figure 5c). Quantitative immunoblots indicated that the relative acetylation level of LSD1 was not statistically altered by HDAC5 siRNA in MDA-MB-231 cells (Figure 5d). Acetyl-H3K9 was used as control of substrate and its expression was increased by HDAC5 siRNA (Figure 5c). These results suggest that inhibition of HDAC5 alone is not sufficient enough to increase LSD1 acetylation in breast cancer cells.

Inhibition of HDAC5 reactivates expression of LSD1 target genes
In cancer cells, amplified LSD1 expression is frequently associated with abnormal suppression of key tumor suppressor genes. We next examined whether expression of LSD1 target tumor suppressor genes could be reactivated following HDAC5 inhibition. Loss of expression of cyclin-dependent kinase inhibitor p21 and epithelial marker claudin-7 (CLDN7) has been reported to be associated with an aggressive phenotype of breast cancer. The transcription activity of p21 and CLDN7 has been found to be suppressed by enhanced activity of LSD1 in breast cancer. Transfection of HDAC5 siRNA resulted in significantly increased mRNA expression of p21 and CLDN7 in MDA-MB-231 cells (Figure 5e). Quantitative chromatin immunoprecipitation assays revealed that depletion of HDAC5 decreased occupancy of both HDAC5 and LSD1, and increased enrichment of H3K4me2 and acetyl-H3K9 at the promoters of both genes (Figure 5f). These data suggest that transcriptional de-repression of these genes lies largely in the cooperation between HDAC5 and LSD1 at key active histone marks.

Inhibition of HDAC5–LSD1 axis hinders breast cancer proliferation and invasion
To explore the functional role of the HDAC5–LSD1 axis in regulating breast cancer development, stable knockdown of HDAC5 mRNA (HDAC5-KD) was generated in MDA-MB-231 and MCF10A-CA1a cells by infection with short hairpin RNA (shRNA) lentiviral particles. Similar to the effect of transient inhibition of HDAC5 by siRNA, stable knockdown of HDAC5 expression significantly reduced LSD1 protein expression in two independent HDAC5-KD clones (Figure 6a). Loss of HDAC5 in both clones hindered cell proliferation and colony formation in soft agar (Figures 6b and c). The flow cytometry analysis showed that inhibition of HDAC5 resulted in a greater fraction of cells accumulated at G1 phase and reduction of the S-phase cell fraction (Figure 6d; Supplementary Figure 9). Moreover, loss of HDAC5 attenuated motility and invasion of MDA-MB-231 cells in a Boyden chamber assay (Figure 6e). A rescue experiment indicated that HDAC5 overexpression promoted growth of MDA-MB-231-Scramble cells, but failed to alter the growth of MDA-MB-231-LSD1-KD cells (Figure 6f). An additional rescue study revealed that LSD1 overexpression rescued growth inhibition by HDAC5 depletion in MDA-MB-231-HDAC5-KD cells (Figure 6g). Taken together, these results demonstrate that tumor-promoting activity of HDAC5 is dependent on LSD1 activity in breast cancer cells.

Overexpression of HDAC5 promotes mutagen-induced tumorigenic development in MCF10A cells
To address whether enhanced interaction between HDAC5 and LSD1 is a critical epigenetic alteration driving tumorigenic transformation of breast cancer, we generated two MCF10A cell lines overexpressing HDAC5 (MCF10A-HDAC5). Stable overexpression of HDAC5 in MCF10A cells increased LSD1 protein level and promoted cell proliferation of both clones (Figures 7a and b), indicating a growth-promoting role for HDAC5 in MCF10A cells. Inhibition of LSD1 by shRNA significantly hindered MCF10A growth and reversed the growth promotion mediated by HDAC5 overexpression, suggesting that HDAC5 promotes MCF10A growth in an LSD1 dependent manner (Figure 7c; Supplementary Figure 10). To evaluate if MCF10A-HDAC5 cells have altered susceptibility to tumorigenesis, MCF10A-Vector and MCF10A-HDAC5 cells were cultured for 7 months in medium containing 500 ng/ml ICR191. ICR191 generates genomic instability and genetic variability, and has been successfully used to induce epithelial cell transformation in several models including MCF10A. MCF10A-HDAC5 cells were subsequently tested for the capacity of anchorage-independent growth in soft agar for 4 weeks. The soft agar colony formation study demonstrated that ICR191 treatment improved the ability of MCF10A cells to form growing colonies, and overexpression of HDAC5 significantly promoted ICR191-induced colony formation in MCF10A cells (Figure 7d). To determine the role of LSD1 in HDAC5 enhanced tumorigenic transformation induced by ICR191, scramble control and LSD1 shRNA lentivirus particles were infected into MCF10A-Vector or MCD10A-HDAC5 cells, which had been treated with ICR191 for 7 months, and the soft agar growth assays showed that loss of LSD1 in MCF10A-HDAC5 cells significantly abolished cellular ability in colony formation (Figure 7e). A model illustrating the role of HDAC5–LSD1 axis in breast cancer development is proposed based on the above findings (Figure 7f).

DISCUSSION
High levels of HDAC5 have been found to be associated with poor survival in multiple cancer types. Overexpression of HDAC5 has been reported to be a poor prognostic factor in basal-like breast cancer, a subtype with aggressive clinical characteristics. In this study, the IHC analysis showed that breast cancers expressed higher levels of HDAC5 compared to the matched-normal adjacent breast tissue. Importantly, our study found a positive correlation between HDAC5 and LSD1 proteins in breast tumor cell lines and patient tissue specimens. Increased expression of HDAC5 and LSD1 is correlated with higher stage of breast cancer in our exploratory study. These findings suggest that the coordinated overexpression of HDAC5 and LSD1 may serve as potential novel prognostic markers as well as possible therapeutic targets for breast cancer. More robust studies will be necessary to understand the precise role of elevated protein expression levels of HDAC5 and LSD1 in the risk stratification of breast cancer patients.
LSD1 protein stability is controlled by several post-translational modifications such as ubiquitination and methylation. However, the precise mechanism of how LSD1 protein stability is regulated is still not understood. A previous study reported that stable depletion of CoREST facilitated LSD1 degradation in HeLa cells. However, siRNA-mediated knockdown of CoREST alone in breast cancer cells failed to destabilize LSD1 protein, suggesting additional layers of control of LSD1 protein stability are required in breast cancer. In this study, we observed for the first time that LSD1 protein stability is promoted by HDAC5. We further found

Figure 7. Effect of HDAC5 on growth and mutagen-induced tumorigenic transformation in MCF10A cells. (a) pcDNA3.1 or pcDNA3.1-HDAC5 transfected MCF10A cells (clone 1 and 2) were analyzed for protein levels of HDAC5 and LSD1 by immunoblots with anti-HDAC5 and anti-LSD1 antibodies. (b) The crystal violet assay for growth of MCF10A stably transfected with control vector or pcDNA3.1-HDAC5 plasmids. (c) MCF10A-Vector-1 or MCF10A-HDAC5-1 cells were infected with scramble or LSD1 shRNA lentivirus particles for 5 days followed by crystal violet assays for growth. (d) MCF10A cells transfected with pcDNA3.1 or pcDNA3.1-HDAC5 plasmids were treated with dimethyl sulfoxide or 500 ng/ml ICR191 for 7 months followed by soft agar colony formation assays. (e) After treatment with 500 ng/ml ICR191 for 7 months, MCF10A-HDAC5 cells were infected with scramble control or LSD1 shRNA lentivirus particles and soft agar colony formation assay was carried out. (f) Proposed model of the role of HDAC5–LSD1 axis in breast cancer development. Bars represent the means of three independent experiments ± s.d. **P < 0.01, ***P < 0.001, Student’s t-test.
that the HDAC5 domain containing NLS is essential for LSD1–HDAC5 interaction. The NLS element provides docking sites for 14-3-3 chaperone binding and has been shown to be critical for HDAC5 import into the nucleus and the regulation of its repressor activity.\textsuperscript{17,33} Although an in vitro assay demonstrated that HDAC5 reduced LSD1 acetylation, HDAC5 siRNA treatment in breast cancer cells failed to alter acetylation of LSD1 protein. Our in vivo results suggest that LSD1 acetylation is likely regulated by a large complex that may involve additional protein deacetylases or cofactors. Further studies are needed to identify the regulatory complex and clarify the precise role of HDAC5 in regulation of LSD1 acetylation in breast cancer cells.

Our studies revealed that HDAC5 regulates LSD1 via enhancement of the protein stability of deubiquitinase USP28. High expression of USP28 has been found to promote the progression of breast and colon cancers.\textsuperscript{20,34} Importantly, USP28 has been reported to deubiquitinate important tumor growth regulators such as c-Myc and TP53BP1 that are involved in MYC proto-oncogene stability and DNA damage response checkpoint regulation, respectively.\textsuperscript{35,36} Our pilot microarray study revealed that inhibition of the HDAC5–LSD1 axis down-regulates c-Myc expression (data not shown). Sen et al.\textsuperscript{37} recently reported that HDAC5 is a key component in the temporal regulation of p53-mediated transactivation. All of these findings imply an interaction of HDAC5/LSD1 axis and USP28-associated ubiquitin–proteasome system in regulating downstream targets involved in tumor development. USP28 has been well-characterized for its role in promoting tumorigenesis, and thus is a potential candidate target in cancer therapy. Given the current inability to use drugs to directly target USP28-driven cancer proliferation, our study suggests a novel alternative approach of targeting USP28 stability by development of HDAC5-specific inhibitors in cancer.

Our findings provide supportive evidence showing that HDAC5 control of cell proliferation is largely dependent on LSD1 stabilization. Furthermore, in this study, we showed that non-transformed MCF10A cells overexpressing HDAC5 significantly promoted ICR191-induced transformation of MCF10A cells. The overexpressed HDAC5 is consistently associated with upregulated LSD1 protein expression over the entire course of transformation induction. These data indicate that enhanced crosstalk between HDAC5 and LSD1 may represent a critical mechanism contributing to breast tumorigenesis. HDAC inhibitors hold great promise for cancer therapy. Despite the promising clinical results produced by the HDAC inhibitors in treatment of hematological cancers such as T-cell lymphoma, no apparent clinical evidence indicates that HDAC inhibitors work effectively as a monotherapy against solid tumors including breast tumors.\textsuperscript{38–41} From a clinical perspective, our novel findings have significance for design and development of novel combination strategies targeting HDAC5–LSD1 axis as an alternative approach for improvement of therapeutic efficacy of HDAC inhibitors in breast cancer.

As summarized in Figure 7f, we show for the first time that LSD1 protein stability is promoted by HDAC5 through the LSD1 associated ubiquitin–proteasome system, confirming that the regulation of LSD1 by HDAC5 is a post-translational event. Our novel findings also provide supportive evidence that an orchestrated interaction between HDAC5 and LSD1 is a critical epigenetic mechanism to suppress transcriptional activities of important tumor suppressor genes that may contribute to breast cancer development.

**MATERIALS AND METHODS**

Reagents and cell culture conditions

MDA-MB-231, MDA-MB-468, MCF-7, T47D, HCC-202 and SK-BR-3 cell lines were obtained from the ATCC/NCI Breast Cancer SPORE program. MCF10A-parenatal and MCF10A-CA1a cells were gifts from Dr Saraswati Sukumar (Johns Hopkins University). Cells were cultured in growth medium as described previously.\textsuperscript{10,42}

**Tissue microarrays and immunohistochemistry**

Tissue microarrays (US Biomax, Rockville, MD, USA) were stained using LSD1 or HDAC5 antibodies. Standard staining procedure for paraffin sections was used for IHC according to manufacturer’s recommendations (Vector Labs Inc., Burlingame, CA, USA). Monoclonal antibodies were used for detection of LSD1 (1:800; Cell Signaling, Danvers, MA, USA) and HDAC5 (1:100; Santa Cruz, CA, USA). The staining was visualized using diaminobenzidine, and quantitated using IHC Profiler, an ImageJ plugin (National Institutes of Health, Bethesda, MD, USA).\textsuperscript{43} H-scores were calculated as previously described.\textsuperscript{44} The manual scoring of H-scores was also carried out by two breast cancer pathologists.

**Plasmid construction and stable transfection**

Plasmids pcDNA3.1(+)-FLAG, pcDNA3.1(+)−FLAG-HDAC5 and pDZ-FLAG-USP28 were purchased from Addgene (Cambridge, MA, USA), pReceiver-FLAG-LSD1 was obtained from GeneCopoeia (Rockville, MD, USA). A FLAG-tagged ORF cDNA clone for Jade-2 was purchased from GenScript (Piscataway, NJ, USA). pcDNA3-HA-ubiquitin was obtained from Dr Yong Wan (University of Pittsburgh). HDAC5 deletion mutants were engineered into pcDNA3.1(+)-FLAG-HDAC5 by PCR with primers shown in Table S1. HDAC5-Δ2 was constructed by digesting full-length plasmids with SacII from amino acid 61 to 489. Stable transfection was carried out using Lipofectamine 3000 transfection reagent (Life Technologies, Grand Island, NY, USA), and colonies were selected with 800 μg/ml G418.

siRNA and shRNA treatment and stable cell line generation

Pre-designed siRNA and non-targeting scramble siRNA (Santa Cruz) were transfected into cells following the manufacturer’s protocol. Cells were collected 48 h post-transfection for further analysis. Scramble control, LSD1-specific or HDAC5-specific shRNA lentiviral particles (Santa Cruz) were infected into cells according to manufacturer’s protocol. Cells were treated with 10 μg/ml puromycin 72 h after infection. Single colonies were analyzed for expression of LSD1 or HDAC5 via immunoblots.

**RNA extraction and qPCR**

Total RNA extraction and cDNA synthesis used the methods described previously.\textsuperscript{10} Quantitative real-time PCR was performed on the StepOne real-time PCR system (Life Technologies). All of the TaqMan gene expression assays were pre-designed and obtained from Life Technologies.

**Western blotting**

Western blotting was performed as previously described.\textsuperscript{12,45,46} Antibodies used in this study were shown in Supplementary Table S2. Membranes were scanned with Li-Cor BioScience Odyssey Infrared Imaging System (Lincoln, NE, USA).

**Crystal violet and cell invasion assays**

The crystal violet proliferation assays were performed as described in our previous study.\textsuperscript{47} The invasive capability of breast carcinoma cells was tested with Millipore QCM 24-well invasion assay kit (Merck KGaA, Germany) according to manufacturer’s protocol.

**Soft agar colony formation assay**

A total of 1.2% Bacto-agar (BD Biosciences, Franklin Lakes, NJ, USA) was autoclaved and mixed with growth medium to produce 0.6% agar. The mixture was quickly plated and solidified for 45 min. Cells were suspended in 0.6 ml 2× growth medium and mixed gently with 0.6 ml 0.8% agar /medium. Overall 1 ml of cells with 0.4% agar/medium mixture was added onto plate for solidification. Colony formation was examined using stereo microscopy and analyzed (CellSens Dimension, Olympus, Shinjuku, Tokyo, Japan).

**Flow cytometry analysis**

Cells were collected and fixed with 70% ethanol. The cell pellet was then treated with 1% TritonX-100. Cells were subsequently resuspended in 50 μg/ml propidium iodide (Sigma, St Louis, MO, USA) containing RNase (Roche, Indianapolis, IN, USA) followed by analysis on the LSR II XW4400 workstation (BD Biosciences).
Immunofluorescence
After 48 h of transfection, cells were fixed with 4% paraformaldehyde and incubated with primary antibodies (1:250) overnight at 4 °C. After washing, cells were incubated with fluorescence-labeled secondary antibody (1:100). After washing, coverslips were placed on a glass slide using UltraCruz mounting medium (Santa Cruz) before fluorescence microscope examination.

Immunoprecipitation, ubiquitination and protein half-life assays
The cell lysate was obtained by using immunoprecipitation lysis buffer as described previously.48 LSD1 or IgG antibodies were added to cell lysate. Protein G–plus agarose beads (Santa Cruz) or Flag-M2 affinity gel were collected and subjected to IB. HA-Ubiquitin, pcDNA3.1-Flag-HDAC5 or empty vector plasmids were co-transfected into cells for 38 h. Cells were then treated with 10 μM MG132 for 10 h and collected for immunoprecipitation assay with protein G–plus agarose beads. For half-life studies 48 h after transfection with pcDNA3.1-HDAC5 or HDAC5 siRNA, cells were treated with 100 μg/ml cycloheximide and then collected at indicated times for IB.

Protein acetylation assay
The immunoprecipitates of FLAG-M2 agarose from MDA-MB-231 cells overexpressing USP28 or FLAG-tag LSD1 were used as substrates for the protein deacetylation assay. Pull-down of IgG was used as negative control. A total of 0.25 μg of recombinant human GST-tagged HDAC5 protein (Creative BioMart, NY, NY) was mixed with 30 μl immunoprecipitates or 1.5 μg bulk histone at 37 °C for 6 h in a buffer containing 40 mM Tris–HCl (pH 8.0), 2.5 mM MgCl2, 50 mM NaCl, 2 mM KCl, 0.5 mM DTT, 1 mM EDTA and protease inhibitors. The reactions were then subjected to immunoblots with anti-acetyl lysine antibody (EMD Millipore, Billerica, MA, USA). FLAG-tagged USP28 or LSD1 and bulk histone were probed with anti-FLAG antibody or H3 antibody as loading control. Inactive HDAC5-GST protein was used as negative control by heating recombinant protein at 100 °C and then treated with 10 mM MG132 for 10 h and collected for immunoprecipitation assay with protein G–plus agarose beads. For half-life studies 48 h after transfection with pcDNA3.1-HDAC5 or HDAC5 siRNA, cells were treated with 100 μg/ml cycloheximide and then collected at indicated times for IB.

Chromatin immunoprecipitation
Chromatin immunoprecipitation assay was performed as described previously.11 Primary antibodies against HDAC5, LSD1, H3K4me2 and acetyl-H3K9 were used as indicated for immunoprecipitation of the protein–DNA complexes. PCR primer sets used for amplification of precipitated fragments were shown in Supplementary Table S1. Input DNA was used for normalization.

Statistical analysis
Data were represented as the mean ± s.d of three independent experiments. The quantitative variables were analyzed by the two-tailed Student’s t-test. The χ2 study was used to assess the correlation between HDAC5 and LSD1 protein expression by using median H-scores as the cutoff for high- versus low-protein expression. P-value < 0.05 was considered statistically significant for all tests. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
This work is supported by US Army Breast Cancer Research Program (W81XWH-14-1-0237 to YH; W81XWH-14-1-0238 to NED), Breast Cancer Research Foundation (to NED and SO) and UPCI Genomics Core Facility supported by NCI P30CA047904.

REFERENCES


Supplementary Information accompanies this paper on the Oncogene website (http://www.nature.com/onc)