Award Number: DAMD17-02-1-0345

TITLE: Searching the Epigenome for Novel Breast Cancer Tumor Suppressor

PRINCIPAL INVESTIGATOR: Gregory J. Hannon, Ph.D.

CONTRACTING ORGANIZATION: Cold Springs Harbor Laboratory
Cold Spring Harbor, New York 11724

REPORT DATE: September 2003

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Searching the Epigenome for Novel Breast Cancer Tumor Suppressor

Gregory J. Hannon, Ph.D.

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

E-Mail: hannon@cshl.org

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

Approved for Public Release; Distribution Unlimited

Our initial proposal focused on developing technologies to uncover epigenetic changes that contribute to tumor development. Our initial attempts towards developing genome wide approaches to identify new genes silenced by epigenetic mechanisms encountered problems; however, our efforts to exploit epigenetic mechanisms of gene silencing to study tumor suppressor gene function have been very successful (see below). Therefore, as we enter the second year of funding, we plan to capitalize on the success of the latter experiments to refocus our effort. Our new objectives will build upon the unanticipated advances that we have made in the use of RNAi to manipulate gene expression in mouse and human cell systems and in animal models to explore the role of epigenetic modifications in breast cancer progression.

Cancer biology, genetics, synthetic lethality, apoptosis

Unlimited

Unclassified

Unclassified

Unclassified

Approved for Public Release; Distribution Unlimited

Unlimited

Unlimited

Unclassified

Unclassified

Unclassified

12a. DISTRIBUTION / AVAILABILITY STATEMENT
12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
Table of Contents

Front Cover.................................................................1
SF 298.................................................................2
Table of Contents.........................................................3
Introduction.............................................................4
Body.................................................................4
Key Research Accomplishments.................................4
Reportable Outcomes..................................................6
Conclusions.............................................................6
Appendices
Progress Report

Introduction

Our initial proposal focused on developing technologies to uncover epigenetic changes that contribute to tumor development. Our initial attempts towards developing genome wide approaches to identify new genes silenced by epigenetic mechanisms encountered problems; however, our efforts to exploit epigenetic mechanisms of gene silencing to study tumor suppressor gene function have been very successful (see below). Therefore, as we enter the second year of funding we plan to capitalize on the success of the latter experiments to refocus our effort.

Below is a summary of our progress on areas supported by the grant, as well as a brief description of other progress as it pertains to our revised Aims. We also describe our new objectives. Of note, although our experimental focus has changed, our goals have not – they continue to explore the “epi-genome” to uncover genes that contribute to breast and other cancers.

Body

Key accomplishments

1. Control of Cellular Senescence. Cellular senescence is an extremely stable form of cell cycle arrest that limits the proliferation of damaged cells, including cells encountering telomere malfunction or DNA damage. As a consequence, mutations that disable senescence contribute to cellular immortalization and drug resistance in breast epithelial cells and other cell types. Work from our groups and others indicate that the p53 and p16/Rb tumor suppressor pathways are crucial regulators of senescence, but how activation of these pathways leads to a permanent arrest has remained largely unexplored. This year, we described a distinct heterochromatic structure that accumulates in senescent human fibroblasts, which we designated senescence-associated heterochromatic foci (SAHF) (Narita et al., 2003; Narita and Lowe, 2004). SAHF formation coincides with the recruitment of heterochromatin proteins and the Rb tumor suppressor to E2F-responsive promoters, and is associated with the stable repression of E2F target genes. Notably, both SAHF formation and the silencing of E2F target genes depend on the integrity of the Rb pathway, and do not occur in reversibly arrested cells. These results provide the first insights into the effector mechanisms of cellular senescence, and indicate that the process is under epigenetic control. Since we believe that senescence is a potent mechanism of tumor suppression, we are continuing to characterize this program with the goal of identifying new players that may contribute to breast and other cancers.

2. Analysis of the CBX7 oncogene. We are collaborating with David Beach to characterize the in vivo properties of the putative oncogene, CBX7. CBX7 is a member of the polycomb group family that was identified by virtue of its ability to immortalize epithelial cells in culture (Gil et al., 2003). Previous work indicates that Bmi-1, another polycomb group protein that can form a complex with CBX7, is oncogenic when
overexpressed in mice. Moreover, disruption of Bmi-1 leads to stem cell depletion, suggesting Bmi-1 can contribute to stem cell maintenance. To determine whether CBX7 has similar properties, we produced chimeric mice that expressed CBX7 in the hematopoietic compartment. We showed that CBX7 is a potent oncogene in vivo, capable of both initiating tumorigenesis and cooperating with c-myc to accelerate the onset of malignancies (Scott et al, in preparation). We are currently developing short hairpin RNAs (shRNAs) to suppress CBX7 function, and intend to use them to determine whether CBX7 acts as an oncogene by controlling the INK4a/ARF locus and/or influences stem cell maintenance. Since p16INK4a is an important tumor suppressor in breast cancer, we hope these studies will help elucidate how epigenetic control of its expression can influence normal cell function and cancer development.

3. Use of RNAi to dissect tumor phenotypes. The application of RNA interference (RNAi) to mammalian systems has the potential to revolutionize genetics and produce novel therapies. This year we investigated whether RNAi can be used to suppress gene expression in vivo and produce phenotypes in mice. We showed that shRNAs directed against Neil1, a gene involved in DNA repair, could suppress gene expression in transgenic animals leading to a radiosensitive phenotype (Carmell et al., 2003). Furthermore, we used a murine lymphoma model to show that shRNAs against the p53 tumor suppressor could accelerate myc induced lymphomagenesis (Hemann et al., 2003). Interestingly, different p53 shRNAs produced distinct phenotypes in vivo, ranging from benign lymphoid hyperplasias to highly disseminated lymphomas that paralleled the nullizygous setting. In all cases, the severity and type of disease correlated with the extent to which specific shRNAs inhibited p53 activity. Together, our results show that RNAi can stably suppress gene expression in stem cells and reconstituted organs derived from those cells. Moreover, intrinsic differences between individual shRNA expression vectors targeting the same gene can be used to create an ‘epi-allelic series’ for dissecting gene function in vivo.

Based on the above results, we are now applying RNAi technology to study other known or putative tumor suppressor genes in vivo. For example, we examined the possible tumor suppressor properties Bim and Puma, two “BH3-only” pro-apoptotic members of the Bcl-2 family. Bim contributes to apoptosis in several settings, including anoikis and taxol-induced apoptosis in breast epithelial cells and carcinomas, respectively (Renato et al., 2003; Sunters et al, 2003). Puma is a direct p53 target that appears to be an important p53 effector in drug induced apoptosis (Villunger, et al., 2003; Jeffers et al., 2004). Using the shRNA technology and rapid transgenic technologies we have developed, we showed that disruption of either Bim or Puma cooperate with myc during tumorigenesis (Hemann, et al., in preparation). Moreover, we are currently testing whether suppression of Puma or Bim can promote drug resistance (preliminary studies indicate yes) and whether this is agent dependent.

4. RNAi libraries and other new tools. Over the past year, we also have made advances in RNAi expression technology. For example, with funds from a variety of sources, we developed a large-scale resource for RNAi in mammalian cells (Paddison et al., in press). The initial library focused on covering the human genome and
comprises some 30,000 sequence verified constructs. In addition, biochemical studies on the RNAi mechanism have allowed us to demonstrate that each expressed shRNA gives rise to a single, predictable siRNA. Using this information, we can now apply siRNA design rules to greatly increase the success rate for individual shRNAs. We are also exploring contextual requirements for shRNAs to gain entry into the RNAi pathway. In short, these studies have produced two critical insights. First, using design rules, the AVERAGE shRNA suppresses gene expression by more than 80%. Second, 29nt shRNAs are more potent (per mole of transfected RNA) than siRNAs at suppressing gene expression.

We also have developed conditional strategies for shRNA expression. One approach involves the use of Cre recombinase to ablate the shRNA expression construct from the genome (Dickins et al., unpublished). An alternative approach was developed in collaboration with CSHL colleague, Vivek Mittal (Gupta et al., in press) and uses an ecdysone regulated U6 promoter for reversible silencing. These can be combined with targeting strategies which deliver shRNA cassettes to the genome in single copy, either through lentiviral transgenesis (with T. Rosenquist, SUNY, Stony Brook) or via homologous recombination (with A. Mills, CSHL). Although not all of these tools were developed with support from this Idea Award, they put us in an ideal position to undertake our revised objectives.

Reportable outcomes

None

Conclusions

New objectives

In the last year we have achieved substantial advances in developing RNAi technology and applying it to in vivo systems that we could not have been anticipated when our initial Idea Award was submitted. Therefore, we now wish to refocus our aims to capitalize on these advances, and use the support from the award to expand and utilize our ability to design and use shRNAs in mice. Specifically, we propose to develop an shRNA library capable of targeting 1000 cancer relevant mouse genes, which we will make publically available over the next year. We will then use this library to study cancer relevant phenotypes such as tumorigenesis and drug resistance. Based on our revised aims, we envision year two of this grant being consumed by the construction of necessary resources in the form of mouse shRNA clones, optimized vector and shRNA designs, and comparisons of improved technologies in vivo. Year three would represent an opportunity to begin exploration of the use of RNAi in in vivo models of epithelial carcinomas, particularly taking an approach in breast that we have used so effectively in lymphoma, namely the design of genetic mosaic animals in which tissues are composed of engineered stem cells. Such reconstitution models with primary murine breast epithelial cells already exist and tests of RNAi in these models are already underway at CSHL.
Over the past year, we have made tremendous progress, building upon our deepening understanding of RNAi biochemistry, toward building resources for using RNAi to address fundamental questions in cancer biology. We hope to exploit this unique opportunity to optimize our approaches toward the use of RNAi technology in murine systems and to develop the necessary tools for RNAi-based “epi-genetics” in mouse models of human cancer. Our successes in technology development have filtered through basic cancer research, and we would like to use this Idea Award to foster this progress. We realize that this is a departure from the original aims. However, we feel strongly that the use of existing funding for collaborative efforts between the Lowe and Hannon labs will be the most effective means to take advantage of a time-sensitive opportunity. We would be happy to provide a revised statement of work to reflect this shift in our research direction.
Publications relevant to the project


Literature Cited


Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, MacLean KH, Han

GRADUATE GENETICS PROGRAM RETREAT
FRIDAY, JANUARY 16, 2004
COLD SPRING HARBOR LABORATORY GENOME CENTER
500 SUNNYSIDE BLVD., WOODBURY, NY

09:00 - 09:45  Arrival - coffee and bagels available

09:50 - 10:00  Welcome and introductions
   Peter Gergen, Director Graduate Genetics Program

10:00 - 10:30  Genomic copy number analysis in sporadic human genetic disease
   Eli Hatchwell, Cold Spring Harbor Laboratory

10:30 - 11:00  The role of mesd in embryonic development and signal transduction
   Bernadette Holdener (Biochemistry and Cell Biology, Stony Brook)

11:00 - 11:15  Break

11:15 - 11:45  Matrix metalloproteinase-7 in chronic pancreatitis and pancreatic cancer
   Howard Crawford (Pharmacological Sciences, Stony Brook)

11:45 - 12:15  Electron cryomicroscopy of large macromolecular machines
   Huilin Li (Brookhaven National Laboratory)

12:15 - 12:45  Hantavirus regulation of cellular responses
   Erich Mackow (Medicine, Stony Brook)

01:00 - 02:15  Buffet Lunch, Woodbury Cafeteria (students set up posters)

02:15 - 02:45  Responses of host cells to Francisella tularensis, a potential agent of bioterrorism
   Martha Furie (Pathology, Stony Brook)

02:45 - 03:15  Power and precision of likelihood based linkage analysis of complex diseases
   Nancy Mendell (Applied Mathematics and Statistics, Stony Brook)

03:15 - 03:45  Memories of a fly: Information processing in a simple brain
   Josh Dubnau (Cold Spring Harbor Laboratory)

04:00 - 05:30  Poster session / cocktail reception, Woodbury Cafeteria
   Genetics Program students in the 3rd year and beyond

Directions to the Woodbury Genome Center:
Using the Long Island Expressway (495): go to Exit 46 (Sunnyside Boulevard). At the traffic light turn onto Sunnyside Boulevard and drive north (approximately 1/4 mile). You will approach the Genome Research Center’s gate entrance.

Using the Northern State Parkway heading east: go to Exit 38 (Sunnyside Boulevard). Turn onto Sunnyside Boulevard and drive north until you can drive straight no further. You will approach the Genome Research Center’s gate entrance.

When arriving at the security gate call the front desk from the call box and announce that you are there for the Genetics Retreat.
CURRICULUM VITAE

GREGORY J. HANNON

EDUCATION:
1986 Case Western Reserve University, Cleveland, OH B.A., Biochemistry
1991 Case Western Reserve University, Cleveland, OH Ph.D. Mol. Biology

AWARDS
1982-86 Andrew J. Squire Scholar
1986 Phi Beta Kappa
1992-95 Damon Runyon-Walter Winchell Cancer Research Fund Fellowship
1997-00 Pew Scholar in the Biomedical Sciences
2000- Rita Allen Scholar
2002-2005 U.S. Army Breast Cancer Innovator Award
2003 Faculty of 1000

RESEARCH AND PROFESSIONAL EXPERIENCE
2002-present Professor, Cold Spring Harbor Laboratory, NY
2000-2002 Associate Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
1996-2000 Assistant Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
1994-1995 Staff Associate II, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
1986-1992 Ph.D. Thesis, Case Western Reserve University, Cleveland, OH

PUBLICATIONS:
Carmell, M., Xuan, Z., Zhang, M. and Hannon, G. J. (2002) The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance,
and tumorigenesis. Genes & Development 16: 2733-2742.