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TITLE: Role of CREB in CML

PRINCIPAL INVESTIGATOR: Kathleen M. Sakamoto, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of California  
Los Angeles, CA 90095-1752

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TYPE OF REPORT: Annual

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

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| 1. REPORT DATE<br>01-02-2007                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |             | 2. REPORT TYPE<br>Annual |                            | 3. DATES COVERED<br>1 Feb 2006 – 31 Jan 2007 |                                           |
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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            | 5b. GRANT NUMBER<br>W81XWH-06-1-0243         |                                           |
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| 6. AUTHOR(S)<br><br>Kathleen M. Sakamoto, M.D., Ph.D.<br><br>Email: kms@ucla.edu                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |             |                          |                            | 5d. PROJECT NUMBER                           |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            | 5e. TASK NUMBER                              |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            | 5f. WORK UNIT NUMBER                         |                                           |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br><br>University of California<br>Los Angeles, CA 90095-1752                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |             |                          |                            | 8. PERFORMING ORGANIZATION REPORT NUMBER     |                                           |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |             |                          |                            | 10. SPONSOR/MONITOR'S ACRONYM(S)             |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)       |                                           |
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| 13. SUPPLEMENTARY NOTES<br>Original contains colored plates: ALL DTIC reproductions will be in black and white.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            |                                              |                                           |
| 14. ABSTRACT<br>The purpose of this proposal is to understand the molecular pathways regulating Bcr-Abl positive CML cells. We demonstrated that the transcription factor, CREB, is highly expressed in K562 cells and cells from patients with chronic phase CML. This led us to hypothesize that CREB may play a critical role in regulating proliferation of CML cells. To determine whether CREB and CREB-dependent pathways may be bonafide targets for CML therapy, we chose to downregulate CREB using RNA interference. There are two specific aims. In Aim 1, we will test the hypothesis that downregulation of CREB inhibits proliferation and survival of CML cells. In Aim 2, will test the hypothesis that downregulation of CREB inhibits leukemia progression in vivo and in primary CML cells. We have generated CREB shRNA lentivirus and infected primary mouse and human bone marrow stem cells. We have also infected Ba/F3 cells expressing the T315I mutation of Bcr-Abl with and without CREB shRNA and followed leukemia progression in vivo. Our results suggest that CREB is necessary for both normal stem cell proliferation and differentiation, and leukemic progression. |             |                          |                            |                                              |                                           |
| 15. SUBJECT TERMS<br>CREB, RNA interference, CML, signaling                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |             |                          |                            |                                              |                                           |
| 16. SECURITY CLASSIFICATION OF:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES                          | 19a. NAME OF RESPONSIBLE PERSON           |
| a. REPORT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | b. ABSTRACT | c. THIS PAGE             |                            |                                              | USAMRMC                                   |
| U                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | U           | U                        | UU                         | 52                                           | 19b. TELEPHONE NUMBER (include area code) |

**Table of Contents**

**Introduction..... 4**

**Body..... 5**

**Key Research Accomplishments..... 5**

**Reportable Outcomes..... 5**

**Conclusions..... 5**

**References..... 6**

**Appendices..... 6**

## INTRODUCTION

CREB is a transcription factor that regulates proliferation and survival in both hematopoietic and neuronal cells. We found that overexpressed in the blood and bone marrow from patients with chronic phase CML. We hypothesize that CREB and CREB-dependent signaling molecules may be effective targets for CML therapy. To test this hypothesis we have chosen to downregulate CREB using RNA interference.

## BODY

### Statement of Work

#### **Task 1. To test the hypothesis that downregulation of CREB will inhibit the proliferation and survival of CML cells**

These tasks have been accomplished and are following the timeline outlined in the proposal.

- a. We have generated CREB shRNA constructs and generated lentivirus (Months 1 to 3).
- b. We have infected various CML cell lines with lentivirus and characterize expression of CREB by Western Blot analysis and RT-PCR (Months 3 to 4).
- c. We have tested the effects of CREB shRNA on cell proliferation, survival, and apoptosis (Months 4 to 10) and showed that CREB is necessary for proliferation and survival of K562 cells (CML cell line).
- d. We have examined CREB downstream gene expression, using microarray analysis (Months 10 to 12) and have identified 200 genes that are significantly upregulated and downregulated that also have CREB binding sites according to “chip on chip” data published by Marc Montminy.

#### **Task 2. To test the hypothesis that downregulation of CREB inhibits leukemia progression *in vivo* and in primary CML cells**

- a. We have generated CREB shRNA, bcr-abl, and control retrovirus (Months 1 to 3).
- b. We have examined the effects of CREB shRNA retroviral infection on normal stem cells and follow mice for engraftment in bone marrow transplantation assays (Months 3 to 12). We have also shown that primary hematopoietic stem cells require CREB for proliferation and differentiation *in vitro*.
- c. We will infect mouse bone marrow with CREB and bcr-abl, or control shRNA retrovirus and perform bone marrow transplantation assays. Latency and type of leukemia will be characterized (Months 12 to 24).

These experiments are pending. We have also infected Ba/F3 cells containing the T315I mutation that also express the luciferase gene and injected these cells into SCID mice. Ba/F3 T315I mutation cells infected with CREB shRNA have delayed progression of leukemia compared to scrambled shRNA controls.

- d. Experiments yet to do: We will infect primary human CML stem cells with CREB shRNA and follow proliferation in methylcellulose (3 to 12 months).
- e. If time permits, we will transplant primary CML stem cells transduced with CREB shRNA into NOD/SCID mice and follow mice for development of leukemia (6 to 24 months).

So far, our results are novel and have not been previously described.

#### KEY RESEARCH ACCOMPLISHMENTS

1. Demonstration that CREB is required for normal hematopoietic stem cell proliferation and differentiation.
2. Demonstration that CREB is necessary for CML cell proliferation and survival.
3. Demonstration that CREB inhibits leukemia progression of resistant Bcr-Abl cells in vivo.

#### REPORTABLE OUTCOMES

##### ***Papers:***

1. Cheng JC and KM Sakamoto. Novel Technologies in Stem Cells: RNA interference and Stem Cells. Stem Cells, *in press*.
2. Cheng JC, Kinjo K, Wu W, Schmid I, Shankar DB, Stripecke R, Kasahara N, Pellegrini M, Nelson S, and KM Sakamoto. The Requirement for CREB in Normal Hematopoiesis and Leukemogenesis. Manuscript in preparation.

##### ***Abstracts:***

1. KM Sakamoto. Requirement of CREB in normal myelopoiesis and leukemogenesis. Presentation at Myeloid Workshop, American Society for Hematology, Orlando FL, December 2006.
2. Cheng JC, Shankar D, and KM Sakamoto. Requirement of CREB in Normal and Malignant Hematopoiesis. Accepted for poster presentation. American Society for Hematology, Orlando FL, December 2006.

#### CONCLUSIONS

Our results suggest that CREB plays a critical role in normal cells and CML cells. We are continuing to validate the requirement for CREB in primary

CML cells *in vitro* and *in vivo*. These are novel findings and will advance our understanding of normal and malignant hematopoiesis.

REFERENCES: Not applicable.

APPENDICES: Curriculum Vitae and Manuscript by Cheng and Sakamoto, *in press*.

SUPPORTING DATA: See below.

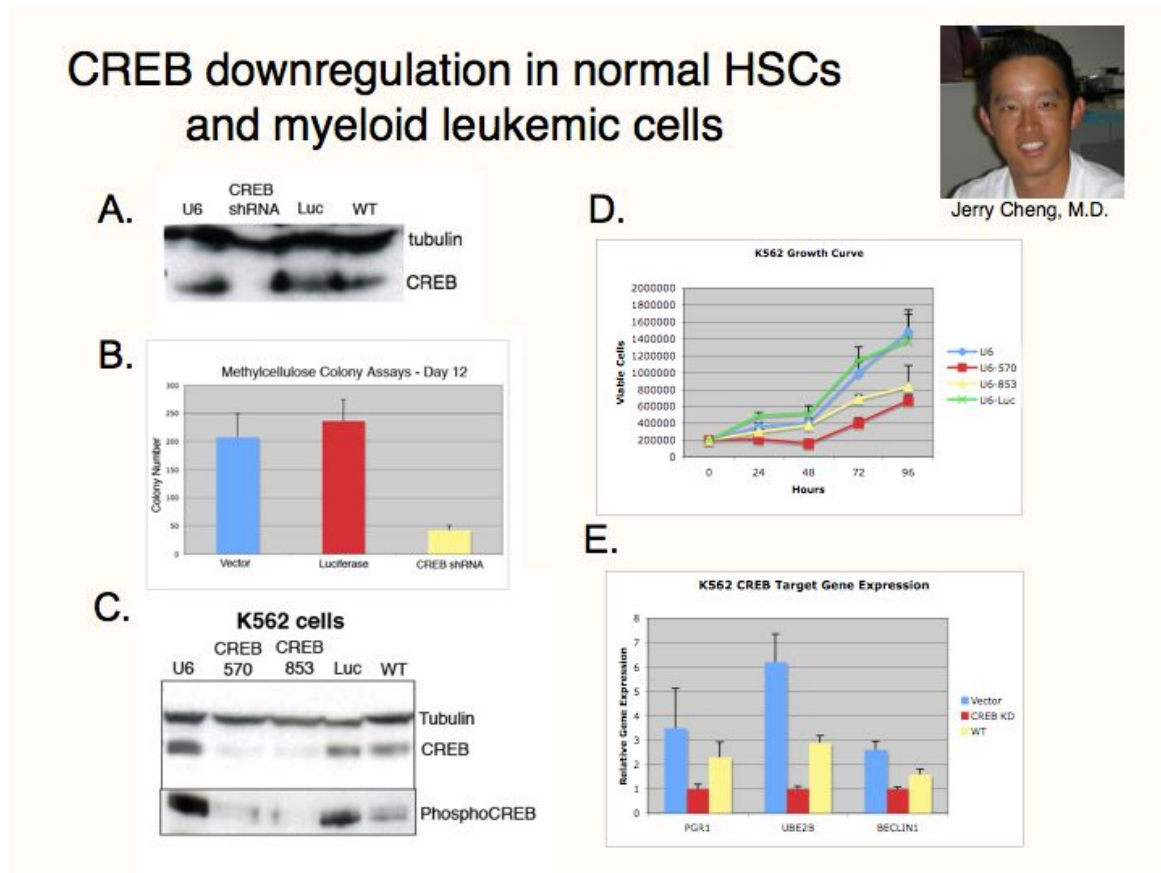


Figure 1. CREB downregulation in normal hematopoietic stem cells (HSC) and CML cells. (A) CREB shRNA lentivirus induces downregulation of CREB by >80%. (B) CREB downregulation results in decreased proliferation and differentiation of normal HSCs. (C) CREB is downregulated in K562 cells infected with CREB shRNA lentivirus. (D) CREB shRNA suppresses K562 proliferation. (E) Candidate target genes in CREB shRNA transduced K562 cells identified using microarray analysis.

## Baf3 cells transduced with bcr-abl and luciferase in mice

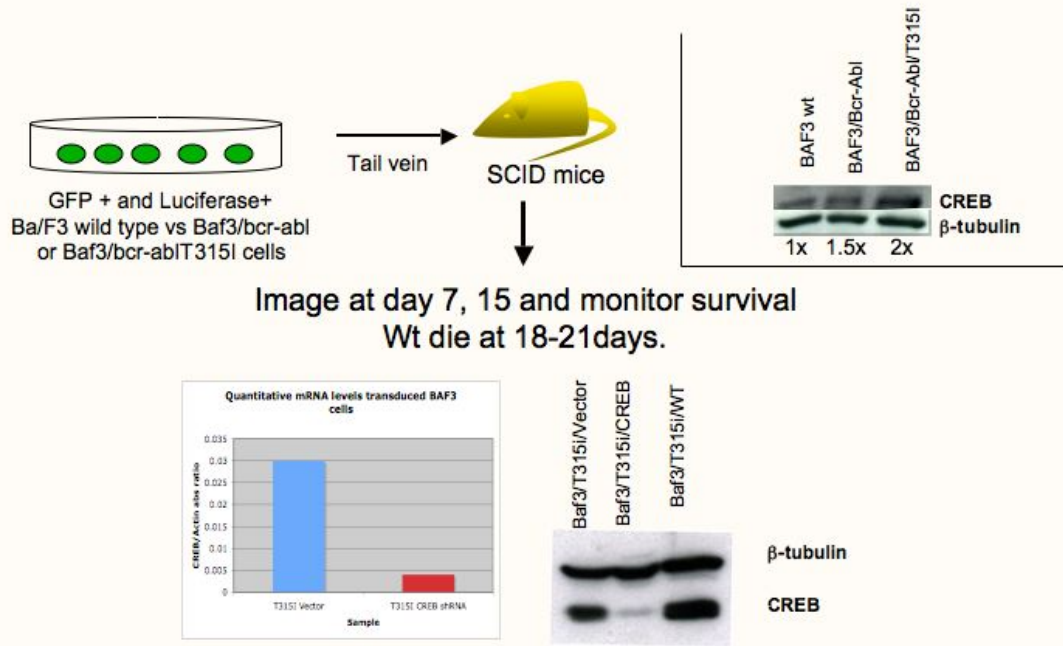
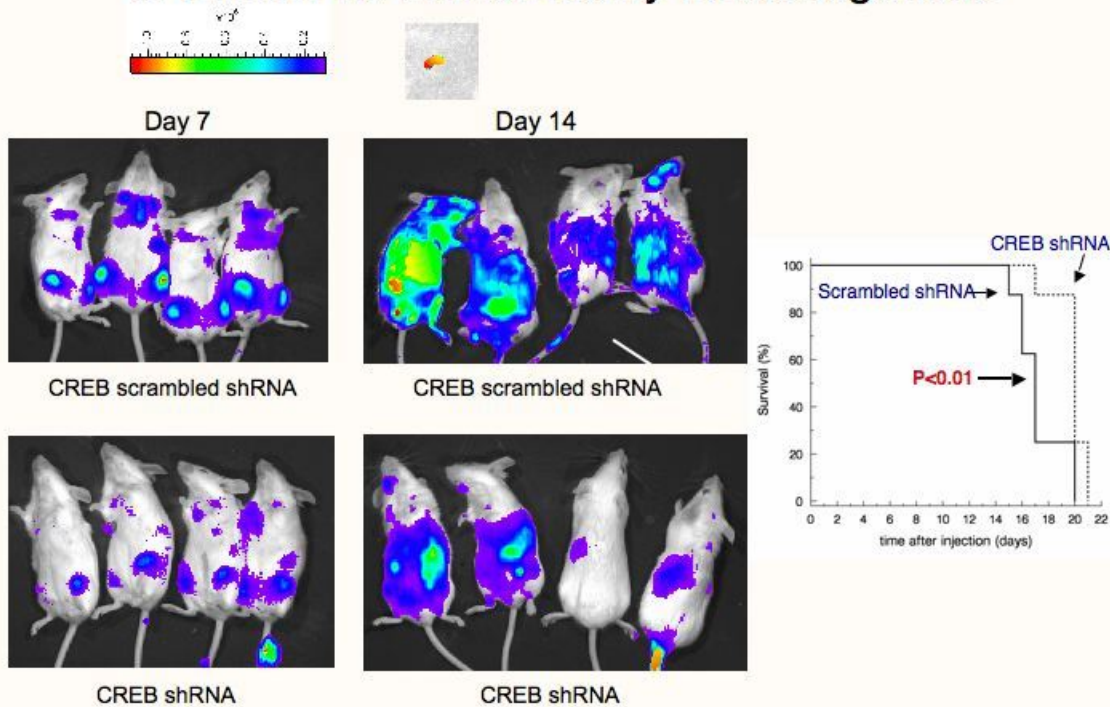


Figure 2. Ba/F3 cells expressing Bcr-Abl T315I mutation. (upper right) CREB is overexpressed in Ba/F3 cells overexpressing Bcr-Abl T315I (Gleevec resistant) mutation by Western Blot analysis. CREB shRNA transduced Ba/F3 cells have significant decrease in CREB expression by quantitative RT-PCR (lower left) and Western blot analysis (lower right).

## CREB shRNA inhibits early leukemogenesis



Cheng et al. ASH #1168, Poster session Saturday Dec 9, 9 am (Board 296-I)

Figure 3. CREB shRNA inhibits early leukemogenesis. (left) Ba/F3 cells that express T315I mutation and luciferase, and transduced with either CREB or scrambled shRNA lentivirus were injected into the tails of SCID mice. Disease progression was followed by bioluminescent imaging. (right) CREB shRNA resulted in a significant decrease in 50% survival of injected mice ( $p < 0.01$ ).

## **CURRICULUM VITAE**

**KATHLEEN MIHO SAKAMOTO, M.D., Ph.D.**

### **CURRENT POSITION**

Vice-Chair, Translational Research  
Professor and Chief  
Division of Hematology-Oncology  
Mattel Children's Hospital  
Department of Pediatrics and  
Department of Pathology and Laboratory Medicine  
David Geffen School of Medicine at UCLA

### **WORK ADDRESS**

Mattel Children's Hospital at UCLA  
Division of Hematology-Oncology  
10833 Le Conte Avenue  
Room A2-412 MDCC  
UCLA School of Medicine  
Los Angeles, CA 90095-1752

### **PHONE NUMBERS**

Phone: 310/794-7007 (office) or 310/206-5626 (lab)  
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### **EMAIL ADDRESSES**

[kms@ucla.edu](mailto:kms@ucla.edu) or [ksakamoto@mednet.ucla.edu](mailto:ksakamoto@mednet.ucla.edu)

### **CITIZENSHIP**

United States

---

### **EDUCATION**

|           |                                                                                                                                       |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------|
| 1975-1979 | Williams College, Williamstown, MA; B.A. Cum Laude; Biology                                                                           |
| 1981-1985 | University of Cincinnati, College of Medicine, Cincinnati, OH; M.D.                                                                   |
| 2000-2003 | California Institute of Technology, Pasadena, CA; Ph.D. Biology<br>Howard Hughes Medical Institute, Laboratory of Raymond J. Deshaies |

### **LICENSURE**

|      |                            |
|------|----------------------------|
| 1986 | California License, G58328 |
| 1994 | DEA license, BS1361992     |

### **BOARD CERTIFICATION**

|      |                                                                                 |
|------|---------------------------------------------------------------------------------|
| 1986 | Diplomate, National Board of Medical Examiners                                  |
| 1989 | Diplomate, American Board of Pediatrics (recertified 1999)                      |
| 1994 | Diplomate, American Board of Pediatrics, Hematology-Oncology (recertified 1999) |

### **RESEARCH INTERESTS**

Growth Factor Signal Transduction  
Leukemogenesis  
Cell Cycle Regulation and Hematopoiesis

### **EMPLOYMENT**

|              |                                                                                                                                |
|--------------|--------------------------------------------------------------------------------------------------------------------------------|
| 1985-1986    | Internship, Pediatrics, Children's Hospital of Los Angeles                                                                     |
| 1986-1988    | Residency, Pediatrics, Children's Hospital of Los Angeles                                                                      |
| 1988-1989    | Fellowship, Hematology/Oncology, Children's Hospital of Los Angeles                                                            |
| 1991-1993    | Clinical Instructor, Division of Hematology-Oncology, Department of Pediatrics, UCLA School of Medicine                        |
| 1993-1994    | Visiting Assistant Professor of Pediatrics, Division of Hematology-Oncology, Department of Pediatrics, UCLA School of Medicine |
| 1994-1998    | Assistant Professor of Pediatrics, Division of Hematology-Oncology, Department of Pediatrics, UCLA School of Medicine          |
| 1995-present | Joint appointment, Department of Pathology and Laboratory Medicine                                                             |
| 1998-present | Associate Professor of Pediatrics and Pathology, Mattel Children's Hospital at UCLA, UCLA School of Medicine                   |
| 2003-present | Professor of Pediatrics and Pathology & Laboratory Medicine                                                                    |
| 2004-present | Visiting Associate, Division of Biology, California Institute of Technology                                                    |
| 2005-present | Professor of Pediatrics and Pathology & Laboratory Medicine, David Geffen School of Medicine at UCLA                           |
| 2005-present | Chief of Division of Hematology-Oncology, Mattel Children's Hospital, David Geffen School of Medicine at UCLA                  |
| 2006-present | Vice-Chair of Translational Research, Mattel Children's Hospital, David Geffen School of Medicine at UCLA                      |

## **RESEARCH EXPERIENCE**

|           |                                                                                                                                                                            |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1978-1979 | Senior Honors Thesis, Department of Biology, Williams College. "Effects of Centrifugation Time on Separation of Plant Organelles".                                         |
| 1979-1980 | Research Assistant, Department of Biochemical Genetics, City of Hope Medical Center                                                                                        |
| 1993-1996 | Research Assistant, Department of Physiology, USC School of Medicine,                                                                                                      |
| 1980-1991 | Postdoctoral Fellow, Division of Hematology-Oncology, in the laboratory of Judith C. Gasson, Ph.D., UCLA School of Medicine                                                |
| 1999      | Visiting Associate, laboratory of Raymond Deshaies, Ph.D., Department of Biology, California Institute of Technology, Pasadena, CA                                         |
| 2000-2003 | STAR program/Graduate Studies, Division of Biology, Laboratory of Raymond Deshaies, PhD, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA |

## **HONORS**

|           |                                                                                                          |
|-----------|----------------------------------------------------------------------------------------------------------|
| 1988      | Victor E. Stork Award, Children's Hospital of Los Angeles                                                |
| 1990-1993 | Leukemia Society of America Fellowship Award                                                             |
| 1991      | Leukemia Society of America award as First Designated Researcher supported by the Leukemia Society staff |
| 1992-1995 | Jonsson Comprehensive Cancer Center/STOP CANCER Career Development Award                                 |
| 1996-2002 | Leukemia Society of America Special Fellow Award                                                         |
| 1994      | Young Investigator Award in Oncology, American Society for Pediatric Hematology-Oncology                 |
| 1995      | UCLA Frontiers of Science Award                                                                          |
| 1996      | Ross Award in Research By Young Investigators (Western Society for Pediatric Research)                   |

|              |                                                                                                                                                                                                 |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1998-2003    | Leukemia Society of America Scholar Award                                                                                                                                                       |
| 1998         | Elected Council Member, Western Society for Pediatric Research                                                                                                                                  |
| 1998         | Participant, AAMC Workshop for Senior Women in Academic Medicine                                                                                                                                |
| 1999         | Invited Participant, American Cancer Society Professors Meeting, October, New York                                                                                                              |
| 1997         | “Meet-the-Expert”, Signal Transduction and Cell Cycle Control in Myeloid Cells, American Society of Hematology, New Orleans, LA                                                                 |
| 1998         | Katherine E. Rogers Scholar for Excellence in Cancer Research, Jonsson Comprehensive Cancer Center, UCLA                                                                                        |
| 1999         | Member of Scientific Review Committee, CONCERN Foundation                                                                                                                                       |
| 2000         | AACR-Novartis Scholar in Training Award, Oncogenomics meeting, Tucson, AZ                                                                                                                       |
| 2001         | Keystone Symposium on “Cell Cycle” - Travel Award, Keystone, CO.                                                                                                                                |
| 2002         | AACR-AFLAC Scholar-in-Training Award, meeting on Ubiquitination and Cancer meeting, Vancouver, Canada.                                                                                          |
| 2002         | Full member, Molecular Biology Institute, UCLA                                                                                                                                                  |
| 2003-present | Children’s Oncology Group, Myeloid Biology Subcommittee                                                                                                                                         |
| 2003-2007    | Member, Grant Review Subcommittee on Leukemia, Immunology, and Blood Cell Development for American Cancer Society                                                                               |
| 2004         | NIH Study Sections on Drug Discovery and Molecular Pharmacology and Basic Mechanisms of Cancer Therapy, and Special Emphasis Panel on Diamond-Blackfan Anemia and Bone Marrow Failure syndromes |
| 2004         | Grant Reviewer, UC Discovery Biotechnology Program                                                                                                                                              |
| 2004         | Moderator, Leukemia Session at American Society for Pediatric Hematology-Oncology Annual Meeting                                                                                                |
| 2004         | Abstract Reviewer and Moderator for “Hematopoiesis: Regulation of Gene Transcription,” ASH Meeting                                                                                              |
| 2005-2009    | Member, NIH Hematopoiesis Study Section                                                                                                                                                         |
| 2004         | Grant Reviewer, Susan G. Komen Breast Cancer Foundation                                                                                                                                         |
| 2005         | “Ask the Experts” in Pediatric Cancer, AACR Public Forum, Anaheim, CA.                                                                                                                          |
| 2005         | Chair of Minisymposium, Modulation of Protein Stability. AACR, Anaheim, CA.                                                                                                                     |
| 2005-present | Member, Translation Research Program Review Subcommittee for the Leukemia and Lymphoma Society of America                                                                                       |
| 2005         | Moderator, Pediatric Hematology-Oncology session, PAS/ASPHO meeting, Washington DC, May 2005                                                                                                    |
| 2005-2009    | Grant Reviewer, California Research Cancer Committee (CRCC)                                                                                                                                     |
| 2005-2009    | Member, ASH Scientific Committee on Myeloid Biology                                                                                                                                             |
| 2006         | Reviewer, NIH Oncology Postdoctoral Fellowship Committee                                                                                                                                        |
| 2006-present | CDMRP (DOD) CML Grant Review Committee                                                                                                                                                          |
| 2006         | ASH abstract reviewer on “Hematopoiesis: Regulation of Gene Transcription” for annual meeting                                                                                                   |
| 2006         | UCLA Finalist, Margaret Early Trust Award                                                                                                                                                       |
| 2006         | Benjamin Franklin High School Wall of Fame Award                                                                                                                                                |
| 2006         | Member, American Pediatric Society                                                                                                                                                              |
| 2007-2008    | Chairman, ASH Myeloid Biology Subcommittee                                                                                                                                                      |

## **EDITORIAL BOARD/REVIEWER**

**Editorial board:** Stem Cells

**Medical Editor,** emedicine online textbook for Pediatrics (Hematology-Oncology section)

**Current Drugs,** panel of evaluators

**Ad hoc reviewer for journals:** Ad hoc reviewer for journals: Blood, Oncogene, Proceedings of the National Academy of Sciences, Molecular and Cellular Biology, Journal of Cellular Biochemistry, Leukemia, Biotechniques, Cytometry, Pediatric Research, Cancer Research, Molecular Cancer Therapeutics, American Journal of Hematology, Molecular Genetics and Metabolism, American Journal of Human Genetics, New England Journal of Medicine, Pediatric Blood and Cancer, Cancer Research, Clinical Cancer Research, British Journal of Hematology, Clinical Prostate Cancer, Pediatrics

## **PROFESSIONAL SOCIETY MEMBERSHIPS**

Candidate Fellow, American Academy of Pediatrics  
Member, American Society of Hematology  
Member, American Society of Pediatric Hematology-Oncology  
Member, American Association for Cancer Research  
Member, New York Academy of Science  
Member, Western Society for Pediatric Research  
Member, Society for Pediatric Research  
Member, International Society for Experimental Hematology  
Children's Oncology Group, AML Strategy Group  
American Society for Biochemistry and Molecular Biology (ASBMB)  
Member, ASPHO Meeting Committee  
Member, ASPHO Career Development Task Force  
Member, ASH Myeloid Biology Subcommittee

## **COMMITTEES**

|              |                                                                         |
|--------------|-------------------------------------------------------------------------|
| 1994         | Search Committee for Director of the Jonsson Cancer Center              |
| 1995         | Search Committee for Nephrology Faculty Appointment                     |
| 1996-1998    | Admissions Committee, UCLA ACCESS program for graduate students         |
| 1996-1999    | Admissions Committee, Medical Student Training Program, UCLA            |
| 1994-present | UCLA Cancer Committee                                                   |
| 1998-1999    | Chair of Tumor Cell Biology ACCESS Affinity Group for Graduate Students |
| 1996-2002    | Western Society for Pediatric Research (WSPR) Council member            |
| 2002-present | Search Committee for Pediatric Pulmonary                                |
| 2002-present | Search Committee for Pediatric Nephrology                               |
| 2002-present | Search Committee for Pediatric Hematology-Oncology                      |
| 2006         | Search Committee for Pediatric Cardiology                               |
| 2006         | Search Committee for Infectious Disease                                 |
| 2006         | Committee for Loan Repayment, Department of Pediatrics                  |
| 2006         | Search Committee for Biostatistician, Department of Pediatrics          |
| 2006-present | Chair, Search Committee for Neonatology                                 |

2006-present              Pediatric Credentials Committee

### **CAMPUS ACTIVITIES**

1994-present              Faculty Mentor on the Medical Student Training Program  
1994-present              Principal Investigator on the Tumor Cell Biology Training Grant  
1995                          Faculty Advisor Program for first year medical students  
1995-present              Principal Investigator on the UCLA ACCESS program for graduate Students

### **Teaching**

1993-present              Pediatric Hematology-Oncology elective  
1993-present              Advanced Clinical Clerkship in Pediatric Hematology-Oncology  
1993-present              Laboratory course in Biochemistry for first year medical students  
1993-present              Pediatric Clerkship  
1993-present              Advanced Clinical Clerkship in Pediatrics  
1995                          Ethics and Accountability in Biomedical Research  
1995-1997                  Major Concepts in Oncology  
1995                          Molecular and Cellular Foundations of Disease  
1993-1997  
conferences                  Organization of Pediatric Hematology-Oncology weekly clinic  
1995-1999                  Organization of the Pediatric Departmental Monthly Research Seminars  
1999-2004                  M229 Course on Cell Biology and Pathogenesis for ACCESS Graduate Students on “Cell Cycle” (organized by Patricia Johnson)  
1996-2003                  Pathophysiology Course in Hematopathology (session on Lymphoma)  
2005-present              Associate Director of the Signal Transduction Program Area, Jonsson Comprehensive Cancer Center  
2005                          MBI 298 seminar course on Ubiquitination  
2005                          Co-organizer, M294 Pathology course on Molecular Basis of Oncology

### **Clinical Activities**

1993-present              Medical Staff, Pediatric Hematology-Oncology, UCLA School of Medicine and Santa Monica Hospital

### **COMMUNITY SERVICE**

Leukemia and Lymphoma Society of America, Los Angeles Chapter, Board of Trustees and Executive Board

### **PATENTS**

“Proteolysis Targeting Chimeric Pharmaceutical” (Raymond Deshaies, Craig Crews, and Kathleen Sakamoto), Ref. No. CIT3284.

“RNA inhibition of CREB” (Jerry Cheng, Kathleen Sakamoto), UC Case No. 2003-348

### **GRANTS**

1989-1990                  American Cancer Society Clinical Oncology Fellowship

|                      |                                                                                                                                                                                                                     |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1990-1993            | 5 F32 CA08974-04 Individual National Research Service Award Molecular Analysis of Target Cell Response to Human GM-CSF (\$102,100); National Cancer Institute (Judith Gasson, Ph.D., P.I.)                          |
| 1996-2002            | Fellowship Award, Molecular Characterization of GM-CSF Action (\$70,000) Leukemia Society of America (Judith C. Gasson, Ph.D., P.I.)                                                                                |
| 1993-1998            | K08 CA59463, Clinical Investigator Award, Molecular Characterization of GM-CSF Action (\$383,400), National Cancer Institute (Judith Gasson, Ph.D. P.I.)                                                            |
| 1993-1996            | 3017-93, Special Fellow Award, Molecular Analysis of GM-CSF Action (\$100,400), Leukemia Society of America (K. Sakamoto, M.D., P.I.)                                                                               |
| 1992-1995            | Career Development Award, Molecular Characterization of GM-CSF Action (\$150,000), STOP CANCER (K. Sakamoto, M.D., P.I.)                                                                                            |
| 1992-1993            | Seed Grant, Mutation Analysis of Structure-Function Relationships of Human GM-CSF Receptor Beta Subunit (\$30,000), Jonsson Comprehensive Cancer Center (K. Sakamoto, M.D., P.I.)                                   |
| 1992-1993<br>Service | Mutation Analysis of Structure-Function Relationships of the Human GM-CSF Receptor Beta Subunit (\$25,000), Southern California Children's Cancer and Couples Against Leukemia (declined) (K. Sakamoto, M.D., P.I.) |
| 1993-1995            | Molecular Regulation of egr-1 by IL-3 and PIXY321 in Myeloid Leukemias (\$100,000), Concern II (K. Sakamoto, M.D., P.I.)                                                                                            |
| 1994                 | The Role of Cyclins in Myeloid Leukemias (\$25,000), Southern California Children's Cancer Service and Couples Against Leukemia (K. Sakamoto, P.I.)                                                                 |
| 1995<br>Signal       | UCLA Academic Senate Award (\$1,500), "Stem Cell Factor Activation of Transduction in Myeloid Leukemic Cells" (K. Sakamoto, M.D., P.I.)                                                                             |
| 1995                 | UCLA Frontiers of Science Award, The Regulation and Functional Role of p55CDC in Myeloid Leukemias (\$28,000) (K. Sakamoto, M.D., P.I.)                                                                             |
| 1995                 | UCLA Prime Faculty Research Award, Molecular Regulation of Myeloid Cell Differentiation (\$25,000) (K. Sakamoto, M.D., P.I.)                                                                                        |
| 1995                 | Seed Grant, The Role of SRE-Binding Proteins During Signal Transduction in Myeloid Leukemias (\$27,000), Jonsson Comprehensive Cancer Center (K. Sakamoto, M.D., P.I.)                                              |
| 1995                 | New Assistant Professor Grant, Transcriptional Regulation of egr-1 by Stem Cell Factor in Myeloid Leukemias (\$35,000), Cancer Research Coordinating Committee (K. Sakamoto, M.D., P.I.)                            |

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| 1995-1997    | Shannon Award, NIH (NCI) 1R55CA68221, Molecular Regulation of Myeloid Cell Differentiation, (\$80,000) (K. Sakamoto, M.D., P.I.)                                                                                      |
| 1996         | Concern II Foundation; Molecular Analysis of IL-3 and PIXY321 Signaling Pathways in Myeloid Leukemias (\$50,000) (K. Sakamoto, M.D., P.I.)                                                                            |
| 1996-2002    | First Award R29CA68221, Molecular Regulation of Myeloid Cell Differentiation, (\$350,000), NIH/NCI (K. Sakamoto, M.D., P.I.)                                                                                          |
| 7/97-6/99    | UC Biotechnology STAR Project, S97-03 "p55Cdc and Cell Cycle Regulation" (\$40,000); Amgen, Inc. and University of California (K. Sakamoto, M.D., P.I.)                                                               |
| 7/98-6/99    | Contract with Eli Lilly, Inc. "Multiple Resistance Genes in Leukemias" (\$32,000), Co-PI with Leonard Rome, Ph.D. (K. Sakamoto, M.D., P.I.)                                                                           |
| 7/98-6/99    | Jonsson Comprehensive Cancer Center Seed Grant, "Use of Low Molecular Weight Heparin in Cancer Patients Receiving Stem Cell Transplants," (\$30,000), Co-P.I. with Dr. Sinisa Dovat, M.D. (fellow)                    |
| 7/98-6/2003  | Leukemia Society of America Scholar Award, 1497-99 "The Role of p55Cdc during Myelopoiesis" (\$350,000), Leukemia Society of America (K. Sakamoto, P.I.)                                                              |
| 1/99-12/2001 | Investigator initiated grant, California Cancer Research Program, "Cell Cycle Control and Cancer" (\$400,000), California Department of Health Services (K. Sakamoto, P.I.)                                           |
| 7/99- 6/2000 | Jonsson Comprehensive Cancer Center Seed Grant, "Development of a Novel Class of Protein-inhibiting Anti-cancer Therapeutics" (\$15,000), K. Sakamoto (P.I.) and Raymond Deshaies (Co-P.I., Caltech)                  |
| 1/2000       | CaPCURE research award, "Development of a Novel Class of Protein-Inhibiting Therapeutics for Prostate Cancer" (\$100,000). Raymond Deshaies (P.I., Caltech), K. Sakamoto, and Craig Crews (Co-P.I., Yale University). |
| 1/99-12/02   | Research Project Grant, "Molecular Analysis of Myeloid Cell Proliferation" (\$300,000); American Cancer Society (K. Sakamoto, P.I.)                                                                                   |
| 8/01-7/03    | UC Biostar, "Targeting the estrogen receptor for Proteolysis", with Celgene, Inc. (\$40,000), K Sakamoto, P.I.                                                                                                        |
| 1/02-12/02   | CaPCURE research award, "Targeting the Androgen Receptor for Degradation in Prostate Cancer" (\$75,000) K.Sakamoto (P.I.), Raymond Deshaies (Co-P.I., Caltech) and Craig Crews (Co-P.I., Yale University).            |
| 6/02-7/03    | National Cancer Coalition, "Signal Transduction and Cell Cycle Analysis in Leukemia" (\$5,000), K. Sakamoto (P.I.).                                                                                                   |

- 1/03-12/06 American Cancer Society, Research Scholar Award. “The role of CREB in Leukemogenesis,” (\$625,000). K. Sakamoto (P.I.).
- 1/03-6/04 Department of Defense, “Targeting the estrogen receptor for ubiquitination and proteolysis in breast cancer,” (\$222,819). K. Sakamoto (P.I.)
- 1/03-12/03 Diamond-Blackfan Anemia Foundation, “AML in Diamond-Blackfan Anemia: Molecular Basis and Therapeutic Strategies,” (\$25,000). K. Sakamoto (P.I.)
- 1/1/03-12/31/04 SPORE grant in Prostate Cancer Research, Seed Grant Award, “Targeting the Androgen Receptor for proteolysis in Prostate Cancer,” \$75,000. K. Sakamoto (P.I.)
- 4/1/03-3/31/04 Stein-Oppenheimer Award, “Targeting the Estrogen Receptor in Breast Cancer,” \$20,000. K. Sakamoto (P.I.)
- 6/1/03-5/30/04 Genomic Exploration Seed Grant, Jonsson Comprehensive Cancer Center, “CREB and Human Leukemias,” \$5,000, K. Sakamoto (P.I.)
- 7/1/03-6/30/04 Susan G. Komen Breast Cancer Thesis Dissertation Award,” \$20,000. K. Sakamoto, R. J. Deshaies (P.I.)
- 1/04-12/07 NIH/NHLBI R01 (HL 75826), “The Role of CREB in Leukemogenesis,” (\$200,000/year). K. Sakamoto (P.I.)
- 9/04-8/06 R21, “Ubiquitination and Degradation in Cancer Therapy,” (\$135,000/year). K. Sakamoto (P.I.)
- 7/04-7/05 Department of Defense, “Identification of small non-peptidic ligands that bind the SCF<sup>beta-TRCP</sup> ubiquitin ligase to target the ER for ubiquitination and degradation (\$75,000). K. Sakamoto (P.I.)
- 7/05-5/07 Fulbright Fellowship/MEC (Spain) postdoctoral fellowship, “Targeting the Androgen Receptor for Ubiquitination and Degradation: A new strategy for Therapy in Prostate Cancer” (\$60,000), K. Sakamoto and R. Deshaies (Co-P.I.).
- 5/05 Boyer/Parvin Postdoctoral Fellow Award (\$5,000), awarded to Deepa Shankar, Ph.D., K. Sakamoto (P.I.)
- 7/05 Stone Research Award (\$1,000) award to undergraduate student Winston Wu, K. Sakamoto (P.I.)
- 7/05-6/07 Department of Defense postdoctoral fellowship, “Targeting the Androgen Receptor for Ubiquitination and Degradation: A New Strategy for Therapy in Prostate Cancer,” (\$80,000), K. Sakamoto (P.I.)
- 10/05-9/05 Diamond Blackfan Anemia Foundation, “ Developing a zebrafish model of Diamond Blackfan Anemia.” \$25,000 (K. Sakamoto and S. Lin, P.I.)

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| 10/05-9/09 | NIH/NHLBI R01 (HL083077), “ Molecular and Cellular Characterization of MPD.” \$225,000/ year (K. Sakamoto, P.I.).                                                           |
| 7/06-6/08  | Department of Defense, “The Role of CREB in CML,” \$45,800/year (K. Sakamoto, P.I.).                                                                                        |
| 7/06-6/08  | F32 HL085013 NRSA (NHLBI), “CREB and Hematopoietic Stem Cells,” awarded to postdoctoral fellow Jerry Cheng, M.D. (K. Sakamoto, P.I.).                                       |
| 7/06-6/08  | NCI T32 CA09056 Tumor Cell Biology Training Grant, “Studies in the Mechanisms of Targeted Therapy for Acute Myeloid Leukemia,” for Alan K. Ikeda, M.D. (K. Sakamoto, P.I.). |
| 10/06-9/09 | Leukemia and Lymphoma Society Translational Research Grant, “Targeting Signaling Pathways in Pediatric AML.” \$200,000/year (K. Sakamoto, P.I. and Ted Moore, co-P.I.).     |
| 1/07-12/12 | NHLBI, “Training in Developmental Hematology.” \$225,000/year (K. Sakamoto, P.I.) [Score 153, waiting for funding decision].                                                |

**TRAINING FACULTY ON THE FOLLOWING TRAINING GRANTS (NIH T32 and K12 Programs)**

Tumor Cell Biology  
Tumor Immunology  
Human and Molecular Development  
Hematology  
Vascular Biology  
Neonatology  
Medical Scientist Training Program (MSTP)  
Gene Medicine  
Stem Cell Research Institute

**TRAINEES**

|              |                                                                                                                              |
|--------------|------------------------------------------------------------------------------------------------------------------------------|
| 1991-1993    | Hu-Jung Julie Lee, undergraduate student                                                                                     |
| 1992-1993    | Elana Lehman, medical student                                                                                                |
| 1993-present | Kathy Hwain Shin, undergraduate student, Work/study and Lab Assistant                                                        |
| 1994-1995    | Robert C. Mignacca, M.D., postdoctoral fellow                                                                                |
| 1994-1995    | Stephen Phillips, undergraduate student, Student Research Project                                                            |
| 1995         | Allison Wong, medical student; Short Term Training Program; Recipient of Howard Hughes NIH Research Scholar Award, 1996-1997 |
| 1995         | Ramona Rodriguez, medical student; Short Term Training Program, Centers of Excellence                                        |
| 1995-2000    | Evelyn Kwon, graduate student                                                                                                |
| 1996         | Michael Mendoza, medical student, Short Term Training Program; of Excellence and FIRST/STAR Award recipient                  |
| Centers      |                                                                                                                              |
| 1996-2002    | Patricia Mora-Garcia (awarded Minority Supplement Award from NIH/NCI), Dept. Pathology and Laboratory Medicine               |

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| 1996-2002    | Michael Lin, graduate students (recipient of NIH/NCI Tumor Cell Biology Training Grant), Dept. Pathology and Laboratory Medicine                                                                                                                                         |
| 1997         | Raymond Wang, medical student, Short Term Training Program                                                                                                                                                                                                               |
| 1995-1999    | Wayne Chu, M.D., Pediatric Resident, Mattel Children's Hospital at UCLA, research elective (recipient of 1999 Merle Carson Lectureship, 1 <sup>st</sup> Prize Southwestern Pediatric Society, The Tenth Joseph St. Geme, Jr. Research Award for UCLA Pediatric Trainees) |
| 1999-2000    | Kristin Baird, M.D. Pediatric Resident, Mattel Children's Hospital at UCLA, research elective                                                                                                                                                                            |
| 2000-present | Deepa Shankar, Ph.D., Postdoctoral fellow (NIH Tumor Cell Biology Postdoctoral fellowship, JCCC fellowship).                                                                                                                                                             |
| 2001-2002    | Heather Crans, graduate student (recipient of NIH Tumor Immunology Training Grant), Dept. Pathology and Laboratory Medicine                                                                                                                                              |
| 2001-2003    | Athena Countouriotis, M.D., Pediatric Resident, Mattel Children's Hospital at UCLA, research elective (recipient of Resident Research Award, American Academy of Pediatrics)                                                                                             |
| 2002-present | Jerry Cheng, M.D., Pediatric Resident, Mattel Children's Hospital at UCLA (won SPR House Officer Award 2003, ASPHO/SPR meeting, Seattle, WA).                                                                                                                            |
| 2002-2003    | Tamara Greene, Medical Student, UCLA School of Medicine                                                                                                                                                                                                                  |
| 2002-2003    | Johnny Chang, M.D., Medical Oncology Fellow, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine (recipient Of NIH Hematology Training Grant)                                                                                               |
| 2003-present | Noah Federman, M.D., Pediatric Resident, Mattel Children's Hospital, research elective                                                                                                                                                                                   |
| 2003         | Andy Liu, undergraduate student (Recipient of Undergraduate scholarship award for research performed in my laboratory)                                                                                                                                                   |
| 2003         | Ryan Stevenson, undergraduate student (now in medical school)                                                                                                                                                                                                            |
| 2004         | Maricela Rodriguez, medical student                                                                                                                                                                                                                                      |
| 2005         | Jenny Hernandez, Saul Priceman, Jose Cordero, Gloria Gonzales, Salemiz Sandoval                                                                                                                                                                                          |
| 2005         | Cid Sumolong, STTP, UCLA medical student                                                                                                                                                                                                                                 |
| 2005-2006    | Winston Wu, undergraduate (recipient of John Stone Award for research performed in my laboratory)                                                                                                                                                                        |
| 2005-present | Salemiz Sandoval, graduate student (MBI)                                                                                                                                                                                                                                 |
| 2005-present | Samuel Esparza, M.D., Pediatric Hematology-Oncology fellow, STAR/PhD graduate program                                                                                                                                                                                    |
| 2005-present | Jerry Cheng, M.D., Pediatric Hematology-Oncology fellow                                                                                                                                                                                                                  |
| 2005-present | Tiffany Simms-Waldrip, M.D., Pediatric Resident                                                                                                                                                                                                                          |
| 2005         | Katrin Rhodes, rotating ACCESS graduate student                                                                                                                                                                                                                          |
| 2006-present | Sam Kaneko, first year UCLA medical student (STTP)                                                                                                                                                                                                                       |
| 2006-present | Kellie Lim, 4 <sup>th</sup> year medical student mentor, UCLA Medical Specialties College Program                                                                                                                                                                        |
| 2006-present | Jenny Hernandez, graduate student (Pathology)                                                                                                                                                                                                                            |
| 2006-present | Alan Ikeda, M.D., Pediatric Hematology-Oncology fellow                                                                                                                                                                                                                   |
| 2006-present | Tara Lin, M.D., Adult Oncology, Postdoctoral fellow                                                                                                                                                                                                                      |
| 2006         | Andrew Goldsmith, ACCESS rotation student                                                                                                                                                                                                                                |
| 2006-present | James Ch'ng, undergraduate student                                                                                                                                                                                                                                       |

## **BIBLIOGRAPHY**

### PEER-REVIEWED

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6. **Sakamoto KM**, Fraser JK, Lee H-J J, Lehman E, Gasson JC: GM-CSF and IL-3 signaling pathways converge on the CREB-binding site in the human EGR-1 promoter. Mol Cell Biol, 14: 5920-5928, 1994.
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through granulocyte-macrophage colony-stimulating factor receptor in BA/F3 cells. Blood, 89:1197-1206, 1997.

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19. **Sakamoto KM**. Clinical Aspects of Childhood MDS. Highlights of the American Society of Pediatric Hematology-Oncology 17<sup>th</sup> Annual Meeting, May 2004. Medscape from WebMD.

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## ABSTRACTS

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44. <sup>1</sup>H. Hsu, <sup>2</sup>N.G. Rainov, <sup>1</sup>F. Sun, <sup>3</sup>**K.M. Sakamoto**, and <sup>1</sup>M.A. Spear. 4-Ipomeanol (4-IM) prodrug activity in cells carrying the p450 CYP4B1 transgene under an EGR1 promoter induced with ionizing radiation. Am. Soc. Ther. Rad. Onc, 1999.

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- \*62. Shankar DB, Chang J, Parcels B, Sandoval S, Li J, Wei R, Tapang P, Davidsen SK, Albert DH, Glaser KB, Moore TB, and **KM Sakamoto**. The Multi-Targeted Receptor Tyrosine Kinase Inhibitor, ABT-869, Induces Apoptosis of AML cells both *in vitro* and *in vivo*. Accepted for an oral presentation at the American Society for Hematology, Atlanta GA, December 2005.
- \*63. Shankar DB, Kinjo K, Chang J, and **KM Sakamoto**. CREB Transgenic Mice Develop Myeloproliferative Disease/Myelodysplastic Syndrome after a Prolonged Latency. Accepted for an oral presentation, American Society for Hematology, Atlanta GA, December 2005.
- \*64. Parcels BW, Ikeda AK, Moore TB, Glaser KB, and **KM Sakamoto**. The Multi-Targeted Receptor Tyrosine Kinase Inhibitor ABT-869 Induces Apoptosis in Baf3 cells expressing the FLT3 Internal Tandem Duplication Mutation. Accepted for an oral presentation, WSPR, Carmel, CA. February 2006.
- \*65. Simms-Waldrup T, Hernandez J, Shankar DB, Moore TB, Shoemaker A, and **KM Sakamoto**. Targeting Bcl-2 in acute myeloid leukemia cells. Accepted for an oral presentation, WSPR Carmel, CA. February 2006.
- \*66. Rodriguez-Gonzalez A, Kim KB, Crews CM, Deshaies RJ, and **KM Sakamoto**. Development of PROTACs to target the estrogen receptor for ubiquitination and degradation in breast cancer cells. Accepted for an oral presentation. AACR meeting, Washington DC, April 2006.
67. Francisco Martinez F, Jimenez F, Machuca C, Villegas H, and **KM Sakamoto**. Transcriptional activation of krox-1 induced by sexual hormones in osteosarcoma cells. Accepted for a poster presentation. American Society for Gene Therapy Baltimore, Maryland. May 2006.
68. Cheng JC, Shankar D, and **KM Sakamoto**. Requirement of CREB in Normal and Malignant Hematopoiesis. Accepted for poster presentation. American Society for Hematology, Orlando FL, December 2006.

69. Esparza SE, Shankar DB, and **KM Sakamoto**. Identification of Meis1 as a Target of CREB in Myeloid Leukemogenesis. Accepted for poster presentation. American Society for Hematology, Orlando FL, December 2006.

70. Sandoval S, Shankar DB, and **KM Sakamoto**. Acceleration of Leukemogenesis in CREB Transgenic mice by Retroviral Insertional Mutagenesis. American Society of Hematology, Orlando FL, December 2006.

\*oral presentation of abstract

#### INVITED PRESENTATIONS

1. **Sakamoto KM**. “Cytokine Signals and Cell Cycle Control During Myelopoiesis” Childhood Leukemia, Biological and Therapeutic Advances. April 17, 1998, Los Angeles, California.

2. **Sakamoto KM**. Serine/Threonine Phosphorylation in Cytokine Signaling Workshop sponsored by the National Cancer Institute. March 30, 1999, Washington, D.C.

3. **Sakamoto KM**. “Signal Transduction Pathways Activated by GM-CSF.” October 29-30, 1999. ACS Professors Meeting, New York.

4. **Sakamoto KM**. “Signal Transduction and Cell Cycle Control in Myeloid Cells” for Meet-the-Experts Breakfast, American Society of Hematology, December 5, 1999, New Orleans, LA.

5. **Sakamoto KM**. CapCURE meeting, September 2000, Lake Tahoe. “Novel Approach to treat Prostate Cancer”

6. **KM Sakamoto and RJ Deshaies**. What SCF Ubiquitin Ligases Are and how they can be used to regulate cancer progression, 4/01

7. **Sakamoto KM**. Bone marrow cells regenerate infarcted myocardium, Journal Club, 4/01

8. **Sakamoto KM**. Acute Leukemia for Pediatric Residents at UCLA School of Medicine, 7/01

9. **Sakamoto KM**. ITP, Olive View Grand Rounds, 8/01

10. **Sakamoto KM**. Childhood Leukemia: causes and treatment. American Cancer Society, Los Angeles Chapter, 10/01

11. **Sakamoto KM**. “The Role of SCF Ubiquitin Ligase in Human Disease: Implications for Therapy.” Caltech Biolunch, March 6, 2002.

12. **Sakamoto KM**. “Targeting Cancer-Promoting Proteins for Ubiquitination and Degradation” Signal Transduction Program Area Seminar, Jonsson Comprehensive Cancer Center, UCLA. August 1, 2003.

13. **Sakamoto KM**. “Development of Approaches to Target Proteins for Ubiquitination and Degradation in Human Disease.” Thesis Defense, Caltech. December 18, 2003.

14. **Sakamoto KM.** “Role of CREB in Human Leukemias.” Gene Medicine Seminar Series. Jan 26, 2004.
15. **Sakamoto KM.** “Childhood Neutropenias.” Pediatric Resident Noon conference. February 4, 2004.
16. **Sakamoto KM,** “The Role of CREB in human leukemias”, Gene Medicine Research Seminar, January 26<sup>th</sup> 2004.
17. **Sakamoto, KM.** “CREB and Acute Myeloid Leukemia,” Leukemia Research Group Meeting, March 4, 2004.
18. **Sakamoto, KM.** “The Role of CREB in Leukemogenesis,” Pediatric Research Seminar, May 20, 2004.
19. **Sakamoto, KM.** Meet the Professors lunch for UCLA ACCESS graduate students. October 6, 2004.
20. **Sakamoto KM.** UCLA Training Program in Tumor Biology. Retreat for UCLA ACCESS graduate students. October 31, 2004.
21. **Sakamoto KM.** “Hematology Jeopardy” Pediatric Noon Seminar, December 13, 2004.
21. **Sakamoto KM.** “Transcriptional Regulators in Normal and Malignant Hematopoiesis,” MBI Noon Seminar, November 30, 2004.
22. **Sakamoto KM.** “Targeting Proteins for Ubiquitination and Degradation in Prostate Cancer” GU SPORE seminar, December 21, 2004.
23. **Sakamoto KM.** “Writing your first NIH grant: an overview,” Pediatric Research Seminar, April 7, 2005.
24. **Sakamoto KM.** “Targeting the Ubiquitin-Proteasome System for Cancer Therapy.” Minisymposium on Modulation of Protein Stability, AACR, Anaheim, CA, April 20, 2005.
25. **Sakamoto KM.** “The Role of CREB in Myelopoiesis.” Myeloid Workshop, Annapolis, MD, 2005
26. **Sakamoto KM.** “The Use of RNA Interference to Study and Treat Human Disease.” Organizer, Cell Biology Methods workshop, PAS/SPR meeting, Washington, D.C., 2005.
27. **Sakamoto KM.** Young Investigators Workshop. American Society of Pediatric Hematology-Oncology meeting, Washington D.C. 2005.
29. **Sakamoto KM.** “Update on Acute Leukemia: Where we’ve been and where we are today.” Pediatric Grand Rounds, Children’s Hospital of Los Angeles. August 19, 2005.
30. **Sakamoto KM.** “Acute Myeloid Leukemia,” Pediatric Resident Rounds, September 21, 2005.

30. **Sakamoto KM and Krasne S.** “Grants Writing Workshop,” ACCESS graduate students, September 26, 2005.
31. **Sakamoto KM.** “COG Spring Meeting Report on Acute Leukemia.” Tuesday 3pm Division Conference, March 28, 2006.
32. **Sakamoto KM.** “Successes and Challenges of Childhood Cancer: Leukemia as a Model.” Life after Childhood Cancer, March 29, UCLA symposium sponsored by the Leukemia and Lymphoma Society of America.
33. **Sakamoto KM.** “The Cancer Problem.” M294 Basic Concepts in Oncology Course, April 3, 2006.
34. **Sakamoto KM.** “Update on Childhood Leukemia.” Pediatric Grand Rounds, Martin Luther King Junior Medical Center.
35. **Sakamoto KM.** “Choosing a Career in Basic Science Research.” Young Investigator Workshop (organizer). ASPHO/SPR meeting. April 30, 2006.
36. **Sakamoto KM.** “RNA interference and Stem Cells,” New Approaches in Stem Cell Technologies Workshop, SPR meeting, San Francisco, California. April 29, 2006.
37. **Sakamoto KM.** “Leukemia.” M294 Basic Concepts in Oncology Course, May 10, 2006.
38. **Sakamoto KM.** “Update on Childhood Leukemias.” Olive View Grand Rounds, May 17, 2006.
39. **Sakamoto KM.** “Leukemia” Pediatric Resident Noon Lecture, June 5, 2006.
40. **Sakamoto KM.** “State of Art lecture on AML/Myelodysplastic Syndromes” for fellows, Division Conference, Tuesday July 18, 2006.
41. **Sakamoto KM.** “Preparation of the RRC Site Visit.” Division Conference, August 29, 2006.
42. **Sakamoto KM.** “Molecular and Cellular Characterization of MPD: The Role of CREB in Myelopoiesis.” NIH/NHLBI grantees meeting on MPD and MDS, November 9, 2006.

A Meeting Report on the workshop, “New Technologies in Stem Cell Research” presented at the Society for Pediatric Research, April 29, 2006. San Francisco, California. In press.

## RNA Interference and Stem Cells

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## **ABSTRACT**

RNA interference (RNAi) is a powerful tool to study gene function, especially in stem cells. Small interfering RNAs (siRNAs) can effectively be introduced either with a vehicle or through viral vectors to transiently or stably inhibit the expression of a particular gene target. Much is known about the optimization of siRNAs and method of delivery in mammalian cells. In this review, we discuss design considerations for siRNAs, methods of delivery, optimization of siRNAs, applications to study genes in stem cells, therapeutic applications, and remaining hurdles. With recent advances in RNAi, it is likely that application of this technology will increase in the future.

## INTRODUCTION

RNA interference (RNAi) describes the inhibition of gene expression by double-stranded RNAs (dsRNAs) developed in the mid 1990's <sup>1</sup>. Guo and Kemphues discovered that sense RNA was as effective as antisense RNA for suppressing gene expression in nematode worms (*Caenorhabditis elegans*) <sup>2</sup>. This was followed by the introduction of dsRNA into worms. When single-stranded antisense RNA and double stranded RNA was introduced into worms, they found that dsRNA was more effective than either strand individual in downregulating genes <sup>1</sup>.

RNAi is a multi-step process that involves the generation of small interfering RNAs (siRNAs) *in vivo* through the activity of the RNase III endonuclease 'Dicer.' The resulting 21- to 23-nucleotide (nt) siRNAs mediate degradation of their complementary RNA <sup>3</sup>. It is now thought that RNAi induces gene silencing through various mechanisms. One is by sequence-specific targeted gene silencing. The second is through translational repression (microRNAs). Finally, it has been reported that RNAi maintains silenced regions of chromosomes <sup>3</sup>.

### Basic mechanisms of RNAi

Long double-stranded RNAs (dsRNAs) are the precursors of the siRNAs that trigger the RNAi effect. When dsRNAs enter cells, they are cleaved by an RNase III-like enzyme known as Dicer into siRNAs (Fig 1). These 21-23 nt siRNAs forms part of a siRNA- protein complex known as RISC (RNA-induced silencing complex), which contains helicase activity that unwinds the two strands

of RNA molecules, allowing the antisense strand to bind the targeted RNA <sup>4-7</sup>. RISC also has endonuclease activity that hydrolyzes the target RNA at the site where it binds the antisense strands. Formation of RISC is critical for mRNA degradation. Therefore, the RISC complex mediates the sequence-specific degradation of the target RNAs that contain homologous sequences to the siRNA.

### **What is a desirable target for RNAi?**

Desirable targets of RNAi include genes that are amplified or overexpressed in cells leading to a specific phenotype. The other is an aberrant protein that is encoded by a dominant mutant allele. Examples include oncogenes that produce transformation in mammalian cells. However, genes that are abundantly expressed or have a prolonged half-life may not be efficiently inhibited. Similarly, genes that are redundant may not be effectively downregulated.

The advantages of RNAi are that the targeted degradation is very specific and can result in variable levels of downregulation such that gene dosage effects can be studied. This technology is much easier, quicker, and less expensive than generating knockout mice. RNAi can also be used to inhibit expression of multiple genes at the same time <sup>8-10</sup>.

### **Design of siRNA**

The use of siRNAs has become a common method of downregulating gene expression to screen gene function in many cell types, including stem cells.

Although long dsRNAs (>30 nt) are effective in suppressing gene expression in plants, *Drosophila*, and *C. elegans*; long dsRNAs are cleaved by Dicer to form siRNAs when introduced into mammalian cells and these siRNAs lead to mRNA degradation. However, in mammalian cells, long dsRNAs activate the interferon response pathway leading to non-specific mRNA degradation. The dsRNA-dependent protein kinase (PKR) is activated, resulting in nonspecific translational inhibition<sup>11, 12</sup>. Therefore, the usefulness of dsRNA in mammalian cells is limited.

In general, 21-23nt siRNAs are too short to activate the non-specific dsRNA response pathway, but are effective in inhibiting the expression of specific targets. There are several limitations of using this technology in mammalian cells. In fungi, plants, and worms, siRNAs can be replicated *in vivo*. In mammalian cells, siRNAs do not prime the synthesis of dsRNA to form additional siRNAs, which may explain why this technology is less effective<sup>9</sup>. Nevertheless, there are several examples in which siRNAs are effective in a variety of mammalian cell types, including stem and progenitor cells<sup>1, 13</sup>.

Optimization of siRNAs in mammalian cells is dependent on several factors. One is the accessibility of the target sequence to the desired mRNA substrate. Previous reports have suggested that selecting a target sequence 100-200 nts away from the translational initiation sequence AUG of the gene is desired<sup>1</sup>. However, successful inhibition of gene expression has also been reported for siRNAs targeting various sequences, including the 3' untranslated region<sup>14</sup>. Targeting of the 3'UTR is also useful if rescue experiments are to be performed. There is no reliable way to predict or identify the ideal sequence for

siRNA. Several reports have suggested that sequences that form the stems of the hairpin siRNAs, the loop size and the sequences at the base of the loop might also affect siRNA-induced gene inhibition. Other determinants include thermodynamic stability; siRNA with lower thermodynamic stability for base pairing at the 5' end of antisense (guide) strand and in the middle of the siRNA were more effective at RNAi than those that had stronger base pairings in these regions due to affects on uptake of guide strand into RISC and enhancing RISC binding to target mRNA.

The sequence of siRNAs should be carefully designed. The number of nucleotides should be between 19-23 nt. The GC content should be between 30 and 50%. The preferred format should be AAN<sub>19</sub>TT. Sequence specificity to at least two nucleotides should be confirmed by Blast comparison of the NCBI GenBank database. Finally, one should query against the SNP database <sup>10</sup>.

### **Optimization of siRNA**

To ensure that the gene of interest is effectively downregulated by the siRNA, it is now recommended that at least three different siRNA sequences per target be designed <sup>15, 16</sup>. More robust knockdown of genes has been reported using this approach of creating “multiplicity” controls. Inhibition of expression has been reported for up to 5 to 10 days when using “pools” of siRNAs in transfected cells.

siRNA concentrations must also be optimized. In general, concentrations of siRNAs greater than 100 nM are considered to be toxic. Various amounts of siRNAs should be tested for each specific cell type. This should be considered when one is using multiple siRNA sequences. Multiple cell lines should also be

tested to validate response and downregulation. Finally, a nucleotide Blast search should be performed to determine whether the siRNA sequence would target another gene. In terms of controls, scrambled or mutated sequence ([www.sirnazizard.com](http://www.sirnazizard.com)), and unrelated genes, e.g. luciferase are commonly used. To validate successful downregulation of the target gene, it is recommended that a Western blot analysis be performed to assess protein levels and Northern blot analysis or RT-PCR to measure RNA levels. Demonstration of lower mRNA levels is critical to rule out a microRNA effect and translational inhibition of gene expression. To control for off target effects, one can measure interferon response genes, including *OAS1*, *OAS2*, and *INFB1* by RT-PCR<sup>1</sup>.

### **Delivery of siRNA to cells**

In mammalian cells, efficiency of siRNA to cells transiently depends on the vehicle or mode of delivery and the cell types. Approaches to introduce siRNAs into cells include a lipid-based vehicle, e.g. lipofectamine, or non-lipid based approach, e.g. calcium phosphate, electroporation. The disadvantages of this approach are that the siRNAs are non-renewable and are only effective as long as they are bath-applied to cells. An alternative strategy has been to deliver siRNAs through a DNA-vector-mediated RNAi approach.

Due to the transient nature of gene silencing produced by oligonucleotide siRNAs and their high costs of chemical synthesis, alternative approaches to introduce siRNAs in plasmid vectors have been developed. A variety of expression vectors are now available. Expression is driven by either the U6 (small nuclear RNA) or H1 RNA polymerase III promoters to drive expression of

sequence-specific short hairpin RNAs (shRNAs) in mammalian cells<sup>2</sup>. These systems are based on the expression of siRNAs either as two separate strands or as a single shRNA. It is thought that the shRNAs are processed by Dicer to active siRNAs *in vivo*<sup>17-19</sup>.

For stable expression in stem cells, the successful delivery has been demonstrated with viral vectors. Various recombinant viral vectors have been developed to deliver shRNAs in mammalian cells<sup>10, 20</sup>. Lentiviral vectors are especially effective. The reasons for this are that lentiviruses have broader tropism and receptor independent delivery. They also have the ability to integrate into the genome for stable gene silencing. Finally, lentiviral transduction and expression of shRNAs do not require cell division for integration into the genome<sup>21</sup>. Lentiviral transduction has been successfully performed in cell lines, mouse hematopoietic stem cells (HSCs), and ES cells<sup>22-24</sup>.

Adenoviral vectors have also been reported to be useful for delivering siRNAs to target cells. This vector system has been used to downregulate genes in liver. However, this vector system has limited utility in stem cells, since low transduction rates have been found in embryonic stem cells (ES) and HSCs. This is most likely due to the fact that the receptor for adenovirus is not highly expressed in stem cells<sup>25</sup>. Similarly, adenoviral-associate vectors (AAV) have been successfully used to deliver RNAi to non-stem cells<sup>1</sup>.

If the stable transfection or transduction of siRNAs results in toxic effects to cells, an alternative approach is to use the inducible expression of shRNAs. The tetracycline/doxycycline regulated form of U6 or H1 promoter has been successfully used. If there is leakiness, other inducible systems such as an

Ecdysone-inducible system, are more tightly regulated with less background. A newer approach has been CRE-lox inducible system<sup>26</sup>. Most recently a doxycycline inducible vector that contains a KRAB domain from one third of zinc finger domains was used in cell lines, mouse ES cells, epithelial breast cancer cells, rat brains, CD34+ cells, and in transgenic mice<sup>27</sup>.

### **Application of RNAi in Stem Cells**

There is now emerging evidence that RNAi can be used to study gene function and for therapeutic application. ES cells are pluripotent stem cells that are derived from the inner cell mass of the 3.5-day-old mouse blastocyst<sup>1, 28</sup>. These cells are desirable models to study the regulation of development and cell lineage commitment and differentiation, since ES cells can give rise to all three germ layers. This system is a powerful tool to study development.

Interestingly, long dsRNA has been used in ES cells, but only when undifferentiated. The reason for this is unknown. In differentiated ES cells, siRNAs have been found to be effective in inhibiting genes such as PU1 and c-EBPa<sup>1</sup>. A variety of other genes have been downregulated in ES cells, such as Shp-2 and Oct-4. Synthetic shRNAs recently have been shown to be efficiently transfected transiently with lipofectamine<sup>29</sup>. More commonly, viral vector systems have been used to transduce genes of interest for stable expression of shRNAs.

HSCs are a self-renewing population of cells in the bone marrow that give rise to all differentiated hematopoietic cells<sup>1</sup>. A number of genes have been targeted using RNAi in HSCs. Growth factor receptor genes, clusters of

differentiation, chemokines, oncogenes (*bcr-abl*), tumor suppressors, HIV genes, globin genes, and RPS19 expression have all been successfully targeted. In most cases, retroviral or lentiviral vector systems were used. Electroporation has been used successfully to introduce dsRNA in HSCs<sup>13</sup>. Lipofectamine has also been reported to effectively transfect oligonucleotide siRNAs into hematopoietic progenitor cells<sup>30</sup>. HSCs that are transduced with shRNAs can then be studied *in vitro* using methylcellulose colony assays or *in vivo* in bone marrow transplantation experiments.

#### NSCs and MSCs

Neural stem cells (NSCs) have also been transduced with shRNAs to downregulate genes. Examples of genes inhibited in NSCs by RNAi are MELK, PPAR $\gamma$  and B27.a genes<sup>31-33</sup>. Mesenchymal stem cells (MSCs) have been studied using both viral and non-viral methods. Genes inhibited using viral vectors were beta-catenin, Msx2, and mecdin<sup>2, 34</sup>. Non-viral liposomal methods to introduce siRNAs into MSCs have been used to inhibit EGFR and Connective Tissue Growth Factor<sup>35, 36</sup>. Recently, a Transfection Microarray Approach was generated in which siRNAs were applied onto slides that are coated with poly-L-lysine and fibronectin. MSCs were then placed on top of the poly-L-lysine and siRNA “sandwich.” Fluorescent microscopy was used to then visualize and quantify the degree of downregulation<sup>37, 38</sup>. A similar approach was used with HeLa cells placed on slides treated with siRNAs, in which cells were then followed “real time” using time-lapse fluorescent microscopy as a high throughput method to screen for genes involved in chromosomal segregation<sup>39</sup>.

## **shRNA libraries**

One of the technological advances in the RNAi field has been the development of shRNA libraries to screen for genes that regulate a specific pathway or biological function. Many of the libraries rely on lentiviral vector-based expression.

Libraries have been used to identify deubiquitinating enzymes<sup>40</sup>, sensitivity to small molecule inhibitors, novel cancer genes, and previously unidentified components of signaling pathways. A recent report from the Broad/MIT group (The RNAi consortium) used an shRNA library with 72,600 clones targeting 10,500 human and 5,300 mouse genes. It is anticipated that the numbers of genes targeted could be as high as 15,000 human or mouse genes. Viruses expressing shRNAs can be transiently or stably transduced into mammalian cells<sup>41</sup>. Genes that are involved in a particular cellular process will be identified through identification of the shRNA clones that block the function of the gene. An inducible shRNA library has also been used recently to identify genes that regulate proliferation or survival of diffuse large B cell lymphoma cells to seek novel targets for therapy<sup>42</sup>.

## **Therapeutic applications of RNAi**

The field of RNAi is advancing at a rapid pace. The application of RNAi as gene therapy is now being realized. In mice, delivery of siRNA to downregulate Fas by hydrodynamic tail injected resulted in protection from fulminant hepatitis<sup>43</sup>. A recent report by Samakoglu et al. has demonstrated that sickle globin gene can be downregulated in CD34+ cells using a lentiviral shRNA, with a concomitant increase in g-globin expression in erythroid specific manner<sup>44</sup>. Another advance

has been the successful RNAi-mediated gene silencing in non-human primates. The first report of systemic delivery of *APOB* siRNA in non-rodent species was recently reported <sup>45</sup>. *APOB* is a component of LDL and regulates the storage and metabolism of cholesterol. A liposomal formulation of *APO-B* siRNAs was intravenously administered into cynomolgous monkeys with effective inhibition of *APOB* levels after 48 hours and 11 days. Plasma levels demonstrated that not only LDL and cholesterol levels were lower than controls but HDL levels were not affected. Although previous success was shown with hydrodynamic tail injection of oligonucleotide siRNAs in rodents, this was the first report of siRNAs successfully targeting a gene in non-rodent models.

### **Remaining challenges**

Although the field of RNAi has rapidly progressed, there are several hurdles that remain before fully applying this technology in humans. The specificity and toxicity of siRNAs must be more rigorously examined. The use of lentiviral vectors in gene therapy has led to insertional mutagenesis and malignancies, which must be overcome. Newer generations of lentiviral vectors are currently being studied. Stability of siRNAs is also problematic for long-term use. However, recent advances in nanotechnology have demonstrated that delivery of siRNAs using nanoparticles has potential in the clinics <sup>46</sup>. Given the advances in the field, it is highly likely that within the next few years, RNAi will become a viable approach to treat human disease.

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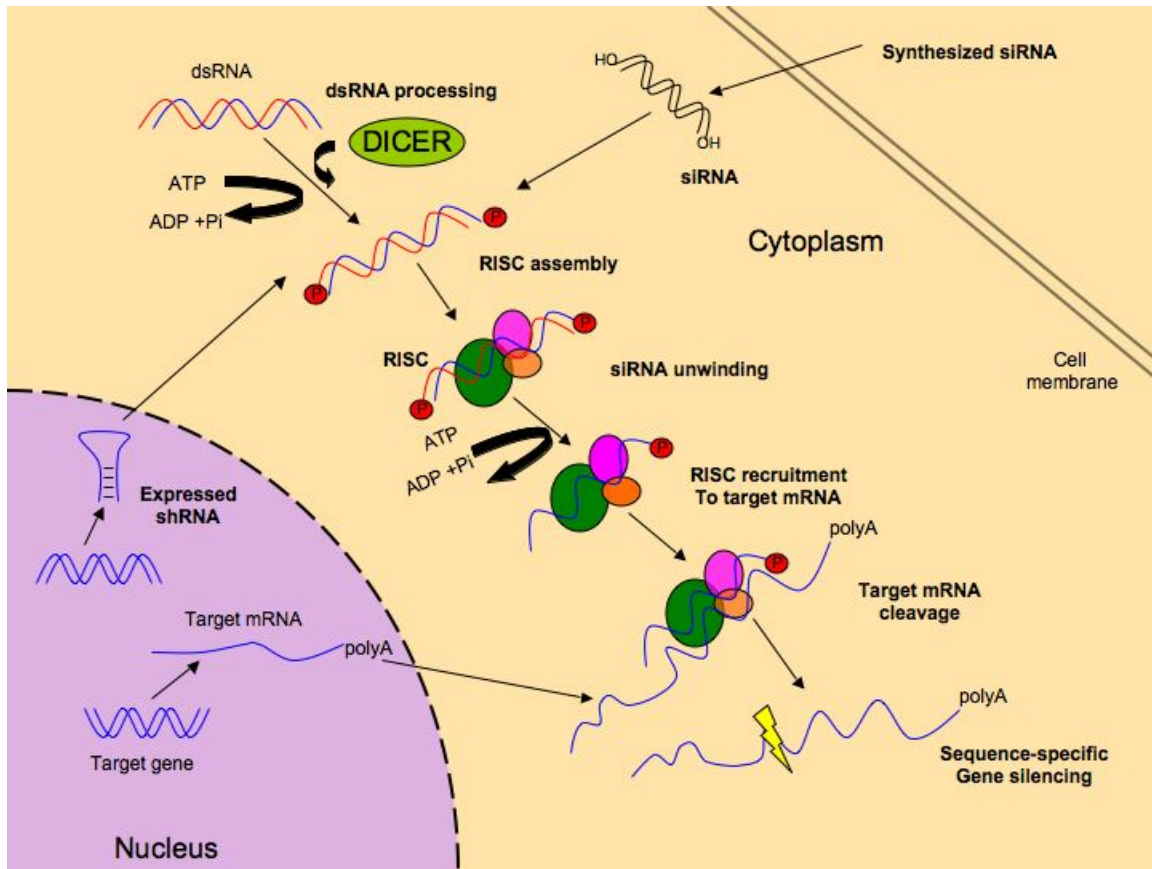
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**Figure 1. siRNA pathways that target mRNA for degradation.**