

AD_____

Award Number: W81XWH-12-1-0082

TITLE: A Genetic Interaction Screen for Breast Cancer Progression Driver Genes

PRINCIPAL INVESTIGATOR: Tian Xu

CONTRACTING ORGANIZATION: Yale University School of Medicine
New Haven, CT 06520

REPORT DATE: June 2013

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE June 2013		2. REPORT TYPE Annual Report		3. DATES COVERED 1 June 2012 - 31 May 2013	
4. TITLE AND SUBTITLE A Genetic Interaction Screen for Breast Cancer Progression Driver Genes				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DOD W81XWH-12-1-0082	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Tian Xu, PhD E-Mail: tian.xu@yale.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Yale University School of Medicine New Haven, CT 06520-8047				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Breast cancer is the most common malignant cancer among American women and the second leading cause of cancer death in women. The analysis of genetic alterations in human breast cancers has revealed that individual tumors accumulate mutations in approximately ninety different genes. However, the significance of most mutations to cancer development remains unknown. The ability to differentiate between driver and bystander mutations will provide valuable insight into how breast cancers develop and how to tailor individualized strategies for the diagnosis and treatment of cancer. We performed a screen to test the roles of seventy breast cancer mutated genes in mouse mammary tumorigenesis using the MMTV-PyVT mouse breast cancer model and <i>piggyBac</i> insertional mutation strains. We found that insertional mutations in 23 genes altered the onset of tumor formation and four genes exacerbated tumor metastasis. Among the 23 genes, <i>Trim33</i> and <i>Ahrr</i> , have been recently reported as tumor suppressors, demonstrating the effectiveness of our screen. We have further confirmed the oncogenic roles of two metabolism related genes, <i>Grik3</i> and <i>HadHB</i> with <i>in vitro</i> tumor assays and are currently performing mechanistic studies. The next phase of questions will be focused on how disruption of metabolism contributes to tumor development and progression.					
15. SUBJECT TERMS Breast cancer, driver mutations, transposon, metabolism					
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	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	6
References.....	6

INTRODUCTION:

Breast cancer is the most common malignant cancer among American women and the second leading cause of cancer death in women. These fatalities are due to the ability of later stage tumors to metastasize to distant sites in the body and form secondary tumors (1, 2). It is now well established that the development of cancer requires multiple genetic alterations (3). In recent years, the systematic analysis of genetic alterations in human cancers has revealed that individual tumors accumulate an average of ninety mutant genes (4). However, the significance of most acquired mutations in the development of cancer remains unknown. A key to deciphering the complexity of the cancer genome is to identify which mutations actually drive tumor development and progression to metastasis. These causative mutations and the pathways they affect are likely the effectors we must target with therapeutics to treat cancer. Thus, novel high throughput strategies are needed to identify and functionally characterize cancer genes. An effective approach to decipher the functions of human genes and test the role of these mutated genes in disease is to systematically mutate the orthologous genes in a mammalian model organism. This approach has not been feasible due to a lack of a large collection of mouse mutant strains. The completed mouse genome sequence has revealed that 99% of human genes have orthologs in the mouse (5). Many developmental processes, signal transduction and regulatory pathways, anatomical structures, and physiological and behavioral characteristics are also well conserved from mice to humans. To facilitate genome scale genetic analysis of human genes and to identify and verify human disease genes, we have initiated a large-scale mouse mutagenesis program at the Institute of Developmental Biology and Molecular Medicine (IDM) at Fudan University, Shanghai, China. This project has led to the establishment of more than 5,000 mutant mouse strains with genetically defined single gene mutations utilizing the *piggyBac* (*PB*) transposon system (6). Among these, we have identified seventy strains for which the orthologous human gene has been found to harbor mutations in breast cancer genome sequencing studies. We performed a screen to test the roles of these seventy genes in mammary tumorigenesis in the MMTV-PyVT transgenic mouse model and here report the preliminary results.

BODY:

Hypothesis: If a gene drives human breast cancer when mutated, then a mouse strain that contains a *PB* insertional mutation in that gene will influence mammary tumor growth and metastasis in the MMTV-PyVT sensitized background.

Objectives:

(1) To use our collection of *PB* mutant mouse strains to identify and characterize which mutations from human breast cancer samples are drivers of tumor initiation and progression.

Using the MMTV-PyVT mouse breast cancer model, we performed a screen to test the roles of seventy breast cancer genes with *PB* insertional mutant mouse lines. Each *PB* mutant mouse line carries a mutant gene corresponding to a human ortholog mutated in human breast cancer. We found that 23 genes altered breast tumor onset and four genes affected metastasis. Among the 23 genes, two of them, *Trim33* and *Ahrr*, have been recently reported as tumor suppressors, demonstrating the effectiveness of our screen (7, 8). We have examined the transcripts affected by the *PB* insertion in these strains and found that sixteen of them have significant reduction of expression while one has increased expression. We then examined the dissected mammary pads and lungs from the MMTV-PyVT/ *PB* insertion mouse strains and confirmed either the enhanced tumor growth or metastasis phenotypes.

(2) To choose four genes from our preliminary screen for follow-up study of the mechanisms underlying their role in breast cancer.

To date we have performed confirmatory experiments with two novel breast cancer genes, *Grik3* and *HadHB*, which have been reported to be involved in metabolism. The *Grik3* gene encodes a subunit of glutamate receptor (also called kainate receptor). We have generated a viral expression system for *Grik3* overexpression. Using this system we demonstrate that overexpression of *Grik3* in NIH3T3-v-Ras cells suppresses cell proliferation and anchorage-independent growth (Figure 1A-B). The kainate receptor is a ligand-gated ion channel which *Grik3* functions to negatively regulate. Accordingly, overexpression of *Grik3* in NIH3T3-v-Ras cells results in a decrease of Ca^{++} concentration within cells and may activate caspase 3 cascade to induce apoptosis (data not shown).

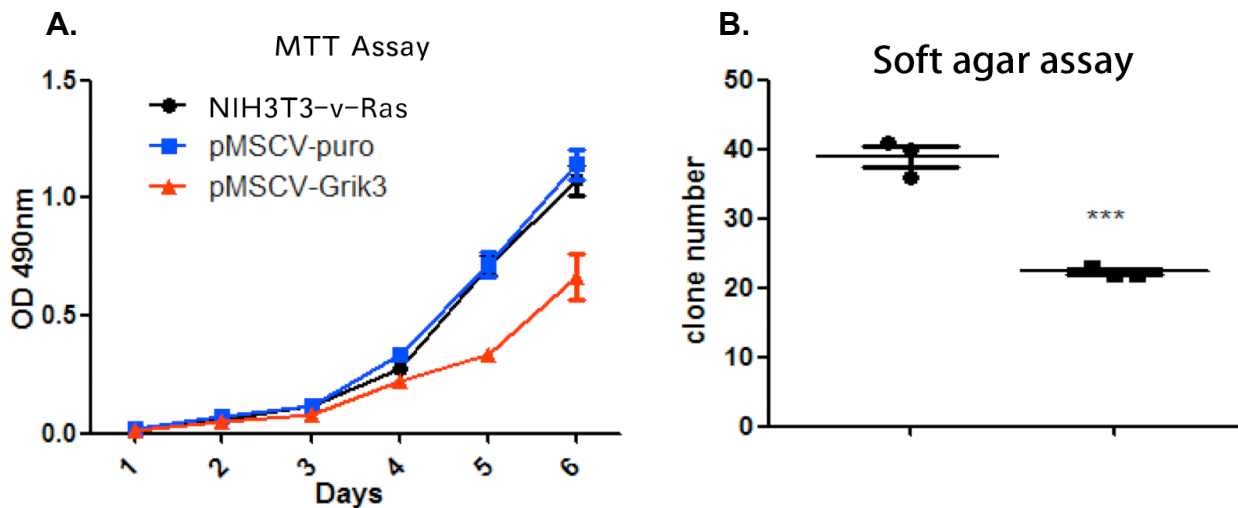


Figure 1. *Grik3* overexpression suppresses oncogenic phenotypes. **A.** Cell number as assayed by MTT assay is plotted over time in NIH3T3-v-Ras parental cells (black circle), NIH3T3-v-Ras pMSCV-puro (blue square), or NIH3T3-v-Ras pMSCV-Grik3 (red triangle) **B.** Average colony number in NIH3T3-v-Ras pMSCV-puro cells (left) or NIH3T3-v-Ras pMSCV-Grik3 (right) in soft agar assay.

HadHB encodes the β -subunit of the mitochondrial trifunctional protein (TFP), which catalyzes the last three steps of mitochondrial β -oxidation of long chain fatty acids. We have also generated a viral expression system for *HadHB* and demonstrated that overexpression of *HadHB* in NIH3T3-v-Ras cells suppresses cell proliferation (data not shown).

We will continue to use tissue culture, biochemistry, and molecular biology experiments to further explore the underlying molecular mechanisms of how these and additional genes identified in the screen drive breast cancer development and progression. The next phase of experiments will focus on how disruption of metabolism contributes to tumor development and progression.

KEY RESEARCH ACCOMPLISHMENTS:

- Screening *PB* mutant mouse strains we identified 23 genes that altered breast tumor onset and four genes that affected metastasis.
- Confirmed that two of the identified genes *Grik3* and *HadHB* demonstrate properties of tumor suppressor genes in tumorigenic assays.

REPORTABLE OUTCOMES:

Animal Models:

We have established *PB* insertional mutation lines for 23 genes that influence tumor onset in the MMTV-PyVT background.

CONCLUSION:

In summary, our *PB* insertional mutation strains have allowed us to rapidly functionally test and identify a collection of novel breast cancer driver genes. Our approach has been validated by the independent verification of two of the identified genes from our screen, *Trim33* and *Ahrr*, by other groups (7, 8). We believe that our innovative approach provides an effective *in vivo* assay to distinguish breast cancer driving mutations from those that are merely bystanders. Consequently, we have verified two additional genes, *Grik3* and *HadHB*, as breast cancer tumor suppressor genes. Both these genes function in cellular metabolism, and thus we may have identified a novel class of breast cancer driver genes. Future work will mechanistically dissect how the disruption of cellular metabolism through loss of these genes drives tumor growth and progression. These experiments should provide important information towards the development of novel therapeutic strategies.

REFERENCES:

1. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. *Nature reviews*. 2009;9(4):274-84.
2. Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: markers and models. *Nature reviews*. 2005;5(8):591-602.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-74.
4. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science*. 2006;314(5797):268-74.
5. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature*. 2002;420(6915):520-62.
6. Sun LV, Jin K, Liu Y, Yang W, Xie X, Ye L, et al. PBmice: an integrated database system of piggyBac (PB) insertional mutations and their characterizations in mice. *Nucleic acids research*. 2008;36(Database issue):D729-34.
7. Herquel B, Ouararhni K, Khetchoumian K, Ignat M, Teletin M, Mark M, et al. Transcription cofactors TRIM24, TRIM28, and TRIM33 associate to form regulatory complexes that suppress murine hepatocellular carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(20):8212-7. Epub 2011/05/03.
8. Zudaire E, Cuesta N, Murty V, Woodson K, Adams L, Gonzalez N, et al. The aryl hydrocarbon receptor repressor is a putative tumor suppressor gene in multiple human cancers. *The Journal of clinical investigation*. 2008;118(2):640-50.