

AWARD NUMBER: W81XWH-15-1-0349

TITLE: Evaluation of alternative splicing regulators as targets for selective therapy of triple negative (basal) breast carcinoma

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REPORT DATE: October 2017

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE October 2017		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2016 - 29 Sep 2017	
4. TITLE AND SUBTITLE Evaluation of alternative splicing regulators as targets for selective therapy of triple negative (basal) breast carcinoma				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0349	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Peter Stoilov E-Mail: pstoilov@hsc.wvu.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) WEST VIRGINIA UNIVERSITY RESEARCH CORPORALAN B MARTIN886 CHESTNUT RIDGEMORGANTOWN WV 26506				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)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
13. SUPPLEMENTARY NOTES					
14. ABSTRACT We failed to generate an antibody specific to SRSF12 (Task 1). This is the second attempt to generate such antibody and we have exhausted the suitable antigenic regions. The SRSF12 antibody is a reagent that is critical for the work related to SRSF12 under Tasks 1, 2, 4, 5, and 6 . Under Task 3 , we completed the backcrossing the of KHDRBS3 and SRPK knockout alleles into the SV129 genetic background and we are generating the experimental animals that combine the transgene with the knockout alleles. Under Task 4 , we analyzed the expression of KHDRBS3 in a breast cancer tissue array, tissue samples from the WVU pathology core and patient derived tumor xenografts. The analysis confirms that KHDRBS3 is associated with triple negative breast cancer. Under Task 5 , we examined the roles of the PI3 kinase pathway, and cMyc on the expression of KHDRBS3. We concluded that KHDRBS3 expression is not controlled by these factors. Tasks 1, 2, and 6 are now complete. The work under these tasks related to SRPK1 and KHDRBS3 is complete, and the work related to SRSF12 is no longer feasible due to the lack of a specific antibody.					
15. SUBJECT TERMS None listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 8	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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Introduction

The goal of this proposal is to evaluate the role of splicing regulators upregulated in triple negative breast cancer in the tumorigenesis process and identify potential therapeutic targets. This will be accomplished through series of experiments in vitro and on animal models of the disease that are designed to determine the effect of splicing factor depletion on tumor initiation, growth and metastasis.

Keywords

Pre-mRNA splicing, breast cancer, KHDRBS3, SRPK1, SRSF12, metastasis

Accomplishments

Major goals

Major Task 1 *Determine the effect of SRPK1, KHDRBS3 and SRSF12 on the malignant properties of the basal type breast cancer cells in vitro.*

Major Task 2 *Determine the effect of SRPK1, KHDRBS3 and SRSF12 on tumor initiation and growth in xenografts.*

Major Task 3 *Demonstrate that reduced SRPK1 and KHDRBS3 levels suppress tumor initiation, growth and lung metastasis*

Major Task 4 *Analyze SRPK1, KHDRBS3 and SRSF12 expression in Splicing factor expression in normal and malignant tissue samples*

Major Task 5 *Analysis of the regulation of splicing factor expression by the PI3K pathway and cMyc*

Major Task 6 *Analysis of the regulation of alternative splicing in mammary basal epithelial cells by SRPK1, KHDRBS3 and SRSF12*

Specific objectives and activities

Major Task 1 *Determine the effect of SRPK1, KHDRBS3 and SRSF12 on the malignant properties of the basal type breast cancer cells in vitro.*

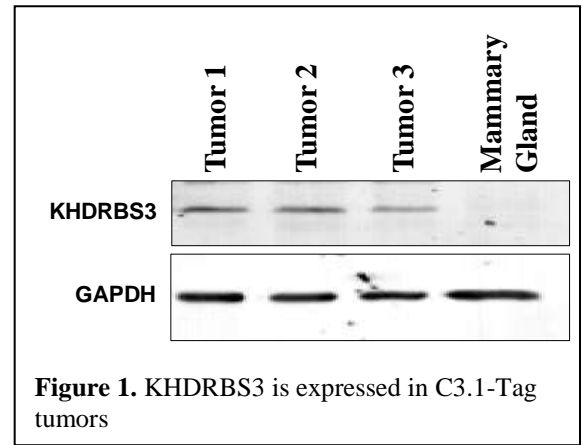
During the reporting period we planned to produce a SRSF12 antibody. During the first year of this project we failed to generate such antibody using the services of Pacific Immunology. We selected a different region of the protein as antigen and used the service provided by Thermo Fisher to generate a polyclonal antibody. This attempt also failed to produce an antibody that would react with recombinant SRSF12 protein. We have now exhausted the regions of the SRSF12 protein that are suitable for producing an antibody that would be selective to SRSF12 and not cross-react with the related SRSF10 protein. Consequently, we lack the means to monitor SRSF12 protein levels and experiments under **Tasks 1, 2, 4, 5, and 6**, that involve SRSF12 cannot be carried out. The work related to SRPK1 and KHDRBS3 under **Task 1** was completed during the previous reporting period. We therefor consider this task to be completed to the extend feasible.

Major Task 2 *Determine the effect of SRPK1, KHDRBS3 and SRSF12 on tumor initiation and growth in xenografts.*

The work related to SRPK1 and KHDRBS3 under **Task 2** was completed during the previous reporting period. Lack of SRSF12 antibody prevents us from carrying out the experiments related to this protein. We therefore consider this task to be completed to the extend feasible.

Major Task 3 *Demonstrate that reduced SRPK1 and KHDRBS3 levels suppress tumor initiation, growth and lung metastasis*

The plan for this reporting period was to backcross the KHDRBS3 and SRPK1 knockout alleles into SV129 background. The backcrosses are now complete and we are now crossing the knockout alleles to the C3.1-Tag transgene. We have already produced KHDRBS3(-/+); C3.1-Tag animals and these are now bred with KHDRBS3(-/-) animals to place the transgene in complete knockout background. Crosses that will establish SRPK1(-/+);C3.1-Tag animals are also under way. We do not plan to produce complete SRPK1 knockouts as these are embryonic lethal. We have produced all necessary control animals that express C3.1-Tag in wild type background and these animals are currently being monitored for tumor formation and progression. Tumor samples were already collected from two control animals in which the tumor burden reached the point that required euthanasia. Preliminary examination of mammary tumors from C3.1-Tag mice shows that KHDRBS3 protein is consistently elevated and the protein is absent from the normal tissue. These data give us confidence that the C3.1-Tag model is suitable for the proposed experiments. We expect this task to be complete in year 3.



Major Task 4 Analyze SRPK1, KHDRBS3 and SRSF12 expression in Splicing factor expression in normal and malignant tissue samples

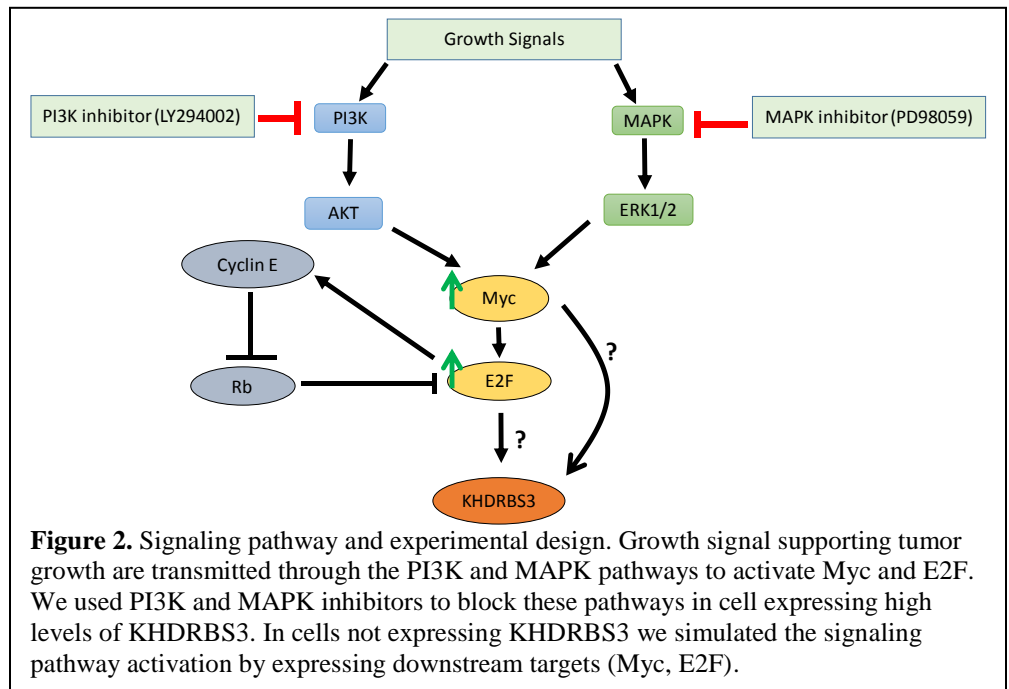
As planned, we used tissue samples from the WVU tissue bank (30 samples) and commercially available breast cancer tissue arrays (181 samples) to analyze the expression of KHDRBS3 in malignant tissue. KHDRBS3 was expressed in 68% of TNBC samples (Table 1). In year 3 of this proposal we will continue to collect breast cancer samples and analyze the KHDRBS3 expression.

Histology status	KHDRBS3 positive	KHDRBS3 negative
ER/PR	10 (20%)	39 (80%)
ER/PR/HER2	9 (20%)	37 (80%)
HER2	11 (30%)	26 (70%)
Triple Negative	20 (68%)	29 (32%)

Table 1. Expression of KHDRBS3 in ductal carcinoma.

Major Task 5 Analysis of the regulation of splicing factor expression by the PI3K pathway and cMyc

We examined the role of PI3K and MAPK pathways in controlling KHDRBS3 expression. Figure 2 shows outline of the experiments. We used inhibitors to block PI3K and MAPK signaling in the MDA-MB-231LN cell that express high levels of KHDRBS3. We also performed the reciprocal experiments where the downstream targets of the two pathways Myc and E2F in MDA-MB-231 cells that express low levels of KHDRBS3. Neither the inhibition of the signaling pathways (Figure 3) nor the expression of the downstream



targets (Figure 4) affected the KHDRBS3 levels. We conclude that KHDRBS3 is not controlled by the PI3K and MAPK pathways. Similar experiments for SRPK1 will be concluded during year 3 of the proposal. Lack of suitable SRSF12 antibodies precludes us from examining its regulation.

Major Task 6 *Analysis of the regulation of alternative splicing in mammary basal epithelial cells by SRPK1, KHDRBS3 and SRSF12*

RNA-Seq experiments during Year 1 of this project demonstrated that KHDRBS3 and SRPK1 have minimal effect on splicing in our cell line models. These results make pointless the planned follow-up experiments. Lack of SRSF12 antibodies prevents us from carrying out gene expression and splicing analysis with this protein as we have no means to monitor the SRSF12 protein levels. The work on this task is now complete.

Major findings

1. Large number of patient tumor samples conclusively demonstrate that the KHDRBS3 protein is expressed preferentially in triple negative cancer.
2. KHDRBS3 expression is not regulated by PI3K and MAPK pathways.

Training and professional development opportunities

Nothing to report

Dissemination of results

Nothing to report

Plans for next reporting period

During the next reporting period we will focus on analyzing the results from the animal models and completing the work on task 5 related to SRPK1. We will continue to collect and analyze patient tumor samples related to

Major task 1. No plans for next reporting period.

Major task 2. No plans for next reporting period.

Major task 3. We will cross our knock-out mouse lines to the C3.1-Tag transgene to produce KHDRBS3(-/-);C3.1-Tag(+), KHDRBS3(+/+);C3.1-Tag(+), SRPK1(+/-);C3.1-Tag(+) and SRPK1(+/+);C3.1-Tag(+) female mice. The goal is to generate 30 mice from each genotype. These mice will be monitored over the course of the year for tumor development. The metastasis to the lungs will be analyzed after the animals have been sacrificed. The monitoring process is expected to complete by the fourth quarter of year 3.

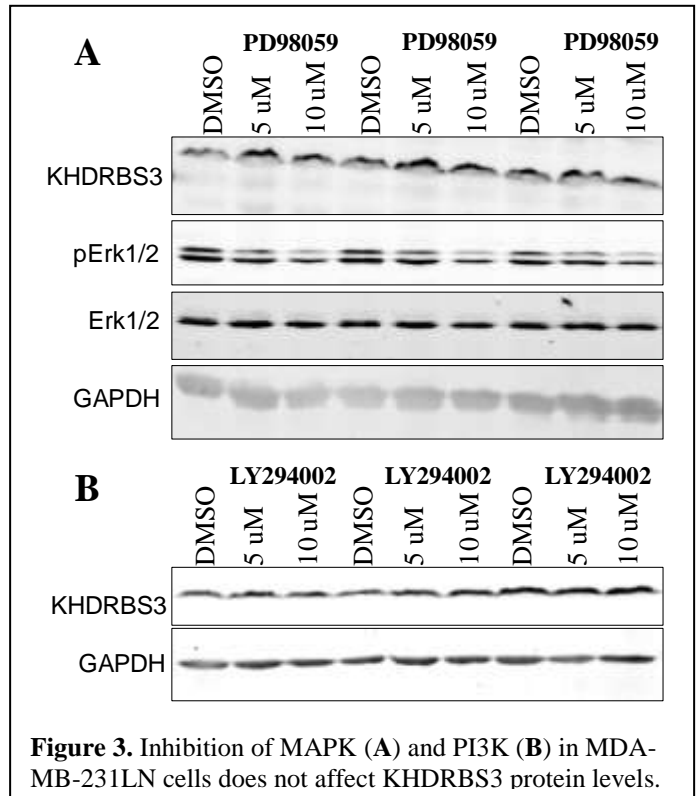


Figure 3. Inhibition of MAPK (A) and PI3K (B) in MDA-MB-231LN cells does not affect KHDRBS3 protein levels.

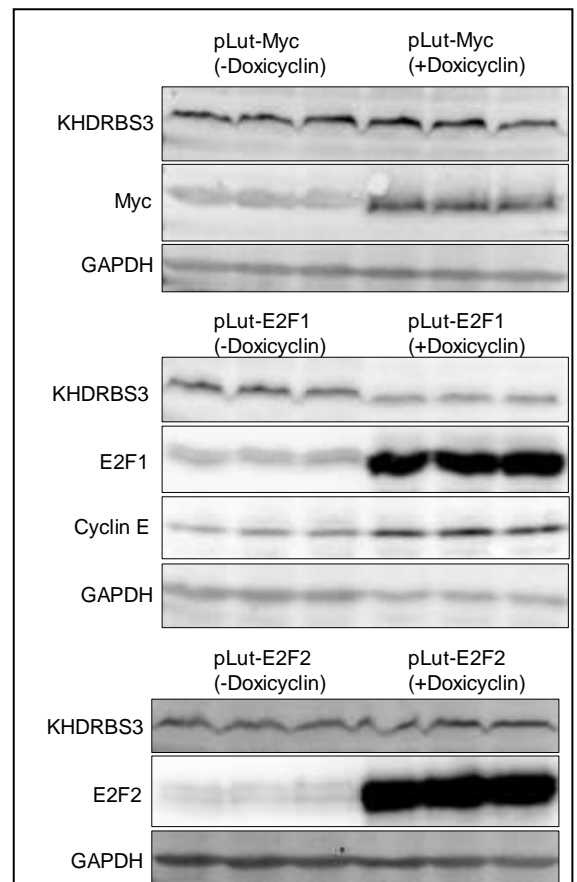


Figure 4. Myc and E2F expression does not affect KHDRBS3 protein levels.

Major Task 4. We will continue to cooperate with the WVU pathology core to analyze KHDRBS3 expression in breast cancer samples collected by the WVU tissue bank.

Major task 5. We will analyze the regulation of SRPK1 expression by the PI3K and MAPK pathways.

Major Task 6. No plans for next reporting period.

Impact

We demonstrated that PI3K and MAPK pathways are not involved in the regulation of KHDRBS3 expression. By analyzing large number of breast cancer samples, we can now conclusively state that KHDRBS3 protein is frequently overexpressed in triple negative breast cancer, but not in other types of breast cancer. To prove the suitability of KHDRBS3 and SRPK1 as targets for drug development, we still need to evaluate their effect on mouse models of breast cancer, which more closely resemble the human disease than cultured cell lines. The actual impact of the current proposal on breast cancer treatment cannot be determined until these experiments are completed.

Changes/Problems

Changes in approach:

1. We are increasing the increasing the animal groups under Task 3 from 10 to 30 animals to deal with higher than expected variability in cancer progression of the C3.1-tag transgenes in the SV129 background (see encountered problems below). No additional funds will be needed to cover the costs for the additional animals and tissue analysis. To cover the expenses, we will use the funds allocated for carrying experiments related to the SRSF12 gene which are no longer feasible due to the lack of working antibody.

Encountered problems:

2. We noted that after crossing into the SV129 background the C3.1-tag transgenes take longer to develop tumors and the age of onset is more variable than in the original B6J background.
3. Despite repeated attempts we were unable to obtain a working antibody to SRSF12. We have exhausted the suitable antigenic regions on the SRSF12 protein and we no longer believe that a selective KHDRBS12 antibody can be produced. As we have no means to monitor the SRSF12 protein levels, experiments related to this protein are no longer feasible.

Products

Nothing to report

Participants & Other Collaborating Organizations

Participants:

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Project Role: Principle Investigator

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Nearest person months worked: 3

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Project Role: Research Assistant

Nearest person months worked: 7

Changes in active other support:

Nothing to report

Other Organizations:

Nothing to report

Special reporting requirements

Nothing to report

Appendices

Nothing to report