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TITLE: Evaluation of Alternative Splicing Regulators as Targets for Selective Therapy of Triple-Negative (Basal) Breast Carcinoma

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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

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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.* ***(Completed in the previous reporting period)***
Major Task 2 *Determine the effect of SRPK1, KHDRBS3 and SRSF12 on tumor initiation and growth in xenografts.* ***(Completed in the previous reporting period)***
Major Task 3 *Demonstrate that reduced SRPK1 and* ***KHDRBS3*** *levels suppress tumor initiation, growth and lung metastasis* ***(Ongoing)***
Major Task 4 *Analyze SRPK1, KHDRBS3 and SRSF12 expression in Splicing factor expression in normal and malignant tissue samples* ***(Ongoing)***
Major Task 5 *Analysis of the regulation of splicing factor expression by the PI3K pathway and cMyc.* ***(Completed in this reporting period)***
Major Task 6 *Analysis of the regulation of alternative splicing in mammary basal epithelial cells by SRPK1, KHDRBS3 and SRSF12* ***(Completed in the previous reporting period)***

Specific objectives and activities

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

Genotype	Number of animals	Animals with breast cancer	Age at which tumor was detected	Age range of tumor free animals
C3.1 Tag+ (control group)	41	8	7-11 months	2-13 months
KHDRBS3+/-; C3.1 Tag+	31	7	6-8 months	7-13 months
KHDRBS3-/-; C3.1 Tag+	30	0	NA	3-10 months
SRPK1+/-; C3.1 Tag+	38	2	7-8 months	2-9 months

Table 1. Status of control and experimental animal groups

In this task we planned to place the oncogenic C3.1-Tag allele in context of Srpk1(+/-), Khdrbs3(+/-) and Khdrbs3(-/-), monitor how reduction of the Srpk1 and Khdrbs3 gene dosage affects tumor formation and analyze the phenotype of the tumors. As the three alleles were originally in different strains we had to carry out back-crossing of at least 5 generations to bring them into uniform genetic background. We have now completed back-crossing the Srpk1, Khdrbs3 and C3.1-Tag alleles to Sv129 genetic background. Power analysis estimated that we will need control and experimental groups of at least 30 animals. During the past year we generated enough animals from each genotype to fulfill the group size requirement (Table 1). The animals are currently being monitored for tumor formation and development. Tumor samples are being collected when the animals are being euthanized for subsequent analysis.

Major Task 4 *Analyze SRPK1, KHDRBS3 and SRSF12 expression in Splicing factor expression in normal and malignant tissue samples*
Nothing to report.

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 Srpk1 expression. Figure 1 shows outline of the experiments. We used inhibitors to block PI3 kinase (PI3K) and MAPK/ERK signaling in the MDA-MB-231LN cell line. Neither the inhibition nor the activation of the signaling pathways affected the Srpk1 protein levels. We conclude that Srpk1 is not controlled by the PI3K and MAPK pathways. This task is now complete.

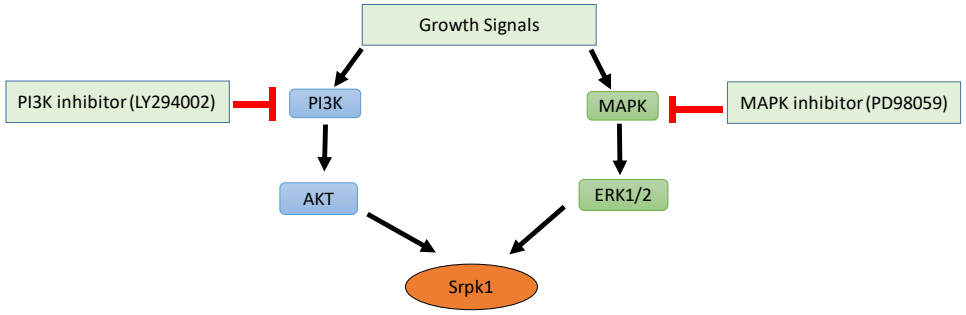

Figure 1. Signaling pathway and experimental design. Growth signal supporting tumor growth are transmitted through the PI3K and MAPK pathways potentially controlling Srpk1 expression. We used PI3K and MAPK inhibitors to block these pathways, or activated them by supplying fetal bovine serum and epidermal growth factor. We then analyzed the effect of these treatments on Srpk1 expression by western blot.

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Major findings
SRPK1 expression is not regulated by PI3K and MAPK pathways.

Training and professional development opportunities
Fatimah Matalkah, who is a technician on this project was accepted in the WVU Health Science Center Graduate program.

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 monitor the animals in the control and experimental groups and collect tumor and tissue (lung, liver) samples until July 2019. The tumor samples will be analyzed for Khdrbs3 and Srpk1 expression by western blot and immunohistochemistry, and compared to adjacent normal tissue.

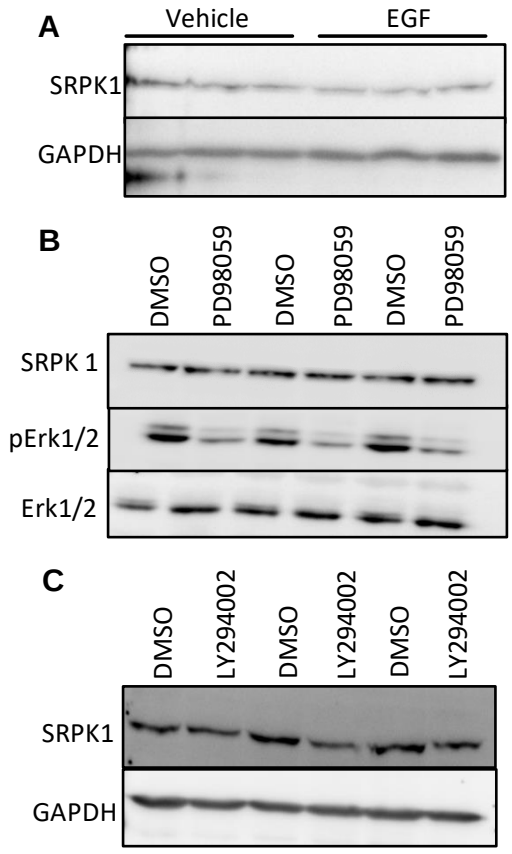

Figure 4. Epidermal Growth Factor stimulation (**A**), inhibition of the MAPK/ERK pathway (**B**) and inhibition of the PI3-kinase/AKT pathway (**C**) does not affect SRPK1 expression. While some changes in SRPK1 levels were observed upon LY294002 treatment, these were not reproducible and we did not detect statistically significant difference.

H&E stained sections from organ samples will be examined for metastasis.
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. No plans for next reporting period.
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:
None

Encountered problems:
1. Delayed onset of tumor formation and difficulties in obtaining the necessary number of experimental and control animals have prevented completion of Major Task 3 in the current reporting period. We now have produced the desired number of animals and the one year no-cost extension will allow us to complete this task.

Products

Nothing to report

Participants & Other Collaborating Organizations

Participants:
Name: Dr. Peter Stoilov
Project Role: Principle Investigator
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Nearest person months worked: 3

Name: Fatimah Matalkah
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