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Award Number: W81XWH-08-1-0197

TITLE: Development and Screening of Subtractive RNAi Libraries from Ovarian Cancer Cell Lines

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REPORT DATE: April 2009

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT:

☒ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-04-2009	Final	01 Apr 2008 – 31 Mar 2009

4. TITLE AND SUBTITLE
Development and Screening of Subtractive RNAi Libraries from Ovarian Cancer Cell Lines

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0197

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Georgetown University
Washington, DC 20057

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 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

The Specific aims of this proposal are:

(1) Construction of two subtractive RNAi libraries targeting mRNA transcripts predominantly present in an (a) advanced ovarian cancer cell line and (b) metastatic ovarian cancer cell line.

(2) Screen for genes vitally important for survival and growth. The two malignant cell lines will be transfected with individual clones from the subtractive RNAi libraries and the effect on viability will be monitored. Clones from the subtractive RNAi libraries producing marked growth inhibition or causing apoptosis will be sequenced and the targeted transcripts identified by BLAST search.

(3) Validation of the identified targets: a) confirmation of the growth inhibitory/apoptotic effect of the selected RNAi clones; b) verification of targeting the translation of the respective mRNAs by Western blot; c) verification of the increased transcription and translation of the target genes in the malignant cell lines by RT-PCR and Western blot.

The proposed studies are expected to be the first, but very important, step towards identification of targets for therapy of advanced and metastatic ovarian cancer. Later the scope of the research will be widened by including samples from patients to confirm the data from the cell lines and eventually, to obtain additional information. The great advantage of our approach, compared to other modern methods (such as microarrays and total transcriptome RNAi libraries) is the relative simplicity and much lower costs.

15. SUBJECT TERMS

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	6	**19b. TELEPHONE NUMBER** *(include area code)*

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Abstract

(1) Construction of two subtractive RNAi libraries targeting mRNA transcripts predominantly present in an
(a) advanced ovarian cancer cell line and
(b) metastatic ovarian cancer cell line.

To this end we will produce cDNA libraries from three cell lines: Hs 832(C)T-benign ovary cyst; Caov-3-primary adenocarcinoma and Caov-4-metastatic adenocarcinoma. The Hs 832(C)T library will be used for subtraction and enrichment for cDNAs highly expressed in the malignant cell lines. The resulting cDNA pools will be used for PCR-based generation of two RNAi libraries: (a) Caov-3 specific and (b) Caov-4 specific.

(2) Screen for genes vitally important for survival and growth. The two malignant cell lines will be transfected with individual clones from the subtractive RNAi libraries and the effect on viability will be monitored. Clones from the subtractive RNAi libraries producing marked growth inhibition or causing apoptosis will be sequenced and the targeted transcripts identified by BLAST search.

(3) Validation of the identified targets.
a) confirmation of the growth inhibitory/apoptotic effect of the selected RNAi clones
b) verification of targeting the translation of the respective mRNAs by Western blot
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The proposed studies are expected to be the first, but very important, step towards identification of targets for therapy of advanced and metastatic ovarian cancer. Later the scope of the research will be widened by including samples from patients to confirm the data from the cell lines and eventually, to obtain additional information. The great advantage of our approach, compared to other modern methods (such as microarrays and total transcriptome RNAi libraries) is the relative simplicity and much lower costs

Introduction

The project was intended to develop a new, relatively simple and cost-and labor-effective, approach to study ovarian cancer by combining a recent method for enzymatic generation of shRNA libraries from cDNA (Ref.1) with an initial step of enrichment for genes specifically expressed in tumor cell lines. The work was expected to result in identification of possible targets for therapy of ovarian malignancies.

Body

In accordance with Work Task 1a mRNA was isolated and converted into cDNA from the following cell lines: IOSE 29, IOSE 80, IOSE592F, Caov-3, SK-OV-3 and OVCAR-3. The IOSE 29, IOSE 80 and IOSE592F cell lines (kind gift from Dr. N.Auersperg, Univ. of British Columbia, Vancouver) were developed by SV-40 large T-antigen transfection of normal ovarian surface epithelium, derived from women without (IOSE 29 and IOSE 80) or with (IOSE 592F)

familial history of ovarian cancer (Ref.2). These cell lines were substituted for the originally planed Hs 832(C)T-benign ovary cyst cell line because they better serve the purpose of enrichment for tumor-specific genes. The rest are widely used ovarian cancer cell lines.

The cDNA prepared form the cell lines IOSE 29 and IOSE 592F was used as driver for subtraction from the testers cDNAs of the ovarian cancer cell lines Caov-3, SK-OV-3 and OVCAR-3. The subtractive hybridization was carried out with the PCR-Select cDNA subtraction kit (Clontech Laboratories, Inc.) according to the manufacturer's manual. In the course of the work some enzymes included in the kit did not perform according to expectation and had to be replaced with products of other suppliers. The efficiency of subtraction was controlled by PCR analysis of the "housekeeping" gene G3PDH cDNA abundance in the subtracted samples. In all of them a significant reduction of the G3PDH cDNA was observed as compared to the unsubtracted control although not to the extent promised by the manufacturer. Still, Work Task 1a was considered as accomplished.

Work Task 1b: an attempt was made to convert the SK-OV-3- and OVCAR-3- enriched subtraction samples (with IOSE 29 and IOSE 592F cDNA used as driver for subtractive hybridization) into shRNA libraries applying the method described by C.Du et al. (Ref.1)

Unfortunately, the effort failed, producing a very low number of clones after two repeats. The same subtracted samples were next subjected to TA- cloning and the result was again not satisfactory: low percentage of clones with insert and insufficient variety in the lengths of the inserts among checked individual clones.

Taking in account the difficulties encountered during performing Work Task 1a and because, to my best knowledge, Clontech is the only supplier of subtraction kits, it was decided to repeat the whole work, starting with Work Task 1a, using only primers, adaptors and PCR kits from Clontech and cDNA synthesis reagents from Invitrogen. In the repeat of the experiments OVCAR-3 cDNA was synthesized and used as tester and IOSE 29 and IOSE 592F cDNA- as driver for subtraction. The consequent attempt to convert the OVCAR-3-specific transcripts enriched cDNA pools to shRNA libraries by the same method did not again produce satisfactory results. Contrary to the expectations I was unable to reproduce the published work with my subtracted cDNA samples. Notably, I am also not aware of other investigators who have successfully used this method for enzymatic conversion of cDNA into shRNA. At this point the Supplies Budget was exhausted and it was close to the end of the allotted time for research, therefore I was not able to try alternative ways to complete Work Task 1b.

On the positive side, both subtracted samples were inserted into TA-cloning vector pCRII with good efficiency. These subtracted libraries will be useful for further studies.

In summary: the aim of Work Task 1b was not achieved and this made it impossible to continue with Work Tasks 2 and 3 as planned.

Key Research Accomplishments

Two subtracted cDNA libraries were produced from the ovarian cancer cell line OVCAR-3. They are enriched for gene transcripts specifically present in the tumor cells. This was achieved by subtractive hybridization with cDNA from two normal ovarian cell lines IOSE 29 and IOSE 592F. Both were developed from normal ovarian surface epithelium cells immortalized by transfection with SV-40 large T-antigen (kind gift from Dr. N.Auersperg, Univ. of British Columbia, Vancouver, Ref.2). The libraries can be used in the future in differential screening for identification of gene products important for the malignant phenotype.

Reportable outcomes

Two subtracted cDNA libraries, which will be useful for further studies.

Conclusion

The project was intended to develop a new approach to study ovarian cancer by combining a recent method for enzymatic generation of shRNA libraries with an initial step of enrichment for genes specifically expressed in tumor cell lines. However, significant difficulties were encountered in the execution of the research plan. In particular, I was unable to reproduce the published method (Ref.1) with my subtracted cDNA samples. The allotted research supplies budget and time frame did not allow for testing other methods for enzymatic conversion of cDNA into shRNA.

On the base of the performed work it is impossible to evaluate the validity of the general research concept. In my opinion it is still a very promising approach provided the above mentioned technical difficulties are resolved.

References

1. Du C, Ge B, Liu Z. et al. 2006. PCR-based generation of shRNA libraries from cDNAs BMC Biotechnology 6:28-38

2. Kruk PA, Godwin AK, Hamilton TC, Auersperg N. 1999. Telomeric instability and proliferative potential in ovarian surface epithelial cells from women with a family history of ovarian cancer. Gynecologic Oncology 73:229-236