

AD\_\_\_\_\_

Award Number: DAMD17-00-1-0566

TITLE: Vitronectin and Integrin  $\alpha v \beta 3$  in Ovarian Carcinoma

PRINCIPAL INVESTIGATOR: Shuang Huang, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute  
La Jolla, California 92037

REPORT DATE: July 2001

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;  
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.

20011212 152

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

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 Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> July 2001	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Jul 00 - 30 Jun 01)	
<b>4. TITLE AND SUBTITLE</b>  Vitronectin and Integrin $\alpha v \beta 3$ in Ovarian Carcinoma			<b>5. FUNDING NUMBERS</b> DAMD17-00-1-0566	
<b>6. AUTHOR(S)</b> Shuang Huang, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  The Scripps Research Institute La Jolla, California 92037  <u>E-Mail: shuang@scripps.edu</u>			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited			<b>12b. DISTRIBUTION CODE</b>	
<b>13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)</b> One hallmark of ovarian cancer is the presence of large amount of floating cells in ascites. However, it is unknown how ovarian cancer cells can survive in absence of adhesion. Our early studies showed that ovarian cancer cells express vitronectin and $\alpha v \beta 3$ integrin on cell surface, and the interaction between them is essential for ovarian cancer cell survival in suspension. In present study, we found that engaging $\alpha v \beta 3$ with vitronectin induced NF- $\kappa$ B activation; Super electrophoresis mobility shift assay with antibodies against individual member of NF- $\kappa$ B family showed that p50 and p65 were in the DNA-protein complex. To determine the importance of NF- $\kappa$ B activity in ovarian cancer cell survival, we blocked NF- $\kappa$ B activity by either expressing dominant negative form of I $\kappa$ B or using NF- $\kappa$ B inhibitors D609 or SN50. In a 2-day growth period, the inhibition of NF- $\kappa$ B activity resulted in over 80% of cell death in ovarian cancer cells cultured in suspension, but did not significantly affect the survival of ovarian cancer cells cultured in adhesion. These results strongly suggest that vitronectin/ $\alpha v \beta 3$ interaction-mediated NF- $\kappa$ B activity is essential for ovarian cancer cell survival in absence of adhesion, and may explain why ovarian cancer cells can survive in suspension.				
<b>14. SUBJECT TERMS</b> $\alpha v$ integrin. vitronectin. cell survival			<b>15. NUMBER OF PAGES</b> 5	
			<b>16. PRICE CODE</b>	
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-5
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	N/A
Appendices.....	N/A

## INTRODUCTION:

The objective of this proposal is to investigate the importance of vitronectin (Vn) and  $\alpha v \beta 3$  integrin in ovarian cancer. The three main goals of this proposal is to 1) examine the mechanism of Vn/ $\alpha v \beta 3$ -mediated ovarian cancer cell survival in suspension culture; 2) to develop potent hammerhead ribozymes targeted against Vn and the  $\beta 3$  integrin subunit; 3) to suppress the efficacy of adenovirus-delivered Vn and  $\beta 3$  integrin subunit-specific ribozymes to suppress tumorigenicity of human ovarian cancer cells in both *in vitro* and *in vivo* experimental models. We believe the better understanding the role of Vn and  $\alpha v \beta 3$  integrin in ovarian cancer cell invasion and survival may lead to a novel therapeutic strategy for ovary malignancies.

## BODY:

We have accomplished all of first year goals (described as Task 1 in our approved Statement of Work).

In the first part of our study, we have measured the effect of engaging  $\alpha v$  integrins with immobilized Vn on NF- $\kappa$ B activity in human ovarian cancer cells. Ovarian cancer OVCAR5 and OVCAR3 cells were transfected with an NF- $\kappa$ B-dependent promoter luciferase reporter gene plasmid, and then added on the culture dishes coated with Vn (2 $\mu$ g/ml) for 2 to 24 hrs. The significant increase in luciferase activity (8-11 fold over the control plasmid-transfected cells) was detected as early as 4 hrs and sustained in all 24 hr period. In a parallel experiment, we also performed electrophoresis mobility shift assay (EMSA). Nuclear extracts were isolated from OVCAR5 and OVCAR3 cells plated on Vn-coated surface for 4 hrs, and then incubated with an oligonucleotide containing NF- $\kappa$ B consensus sequence (Promega) for 30 min. By fractionating the reaction, we found that the mobility of NF- $\kappa$ B consensus sequence-containing oligonucleotide, rather than the mutant NF- $\kappa$ B oligonucleotide, was shifted, further confirming the ability of Vn/ $\alpha v$  integrin ligation to induce NF- $\kappa$ B activity. Furthermore, we also performed super EMSA by incubating nucleus/oligonucleotide complex with antibodies to specific member of NF- $\kappa$ B factor. We found that NF- $\kappa$ B members, p65 and p50, were involved in  $\alpha v \beta 3$  integrin-mediated NF- $\kappa$ B activity. To determine which  $\alpha v$  integrin mediates induced NF- $\kappa$ B activity, we pretreated cells with function-blocking mAb to  $\beta 1$  integrin (P4C10),  $\alpha v \beta 3$  (LM609) or  $\alpha v \beta 5$  (P1F6) prior to adding cells on Vn-coated surface. We found that Vn-induced NF- $\kappa$ B activity is mediated by  $\alpha v \beta 3$  integrin rather than  $\alpha v \beta 5$  or  $\alpha v \beta 1$  integrin since only function-blocking monoclonal antibody to  $\alpha v \beta 3$  was capable of abrogating Vn-induced NF- $\kappa$ B activity.

In the second part of our study, we determined the importance of NF- $\kappa$ B activity in ovarian cancer cell survival in suspension culture. We prepared a recombinant adenoviral vector containing dominant negative I $\kappa$ B gene under tetracycline promoter

control (Ad.tet.IκBm) and tetracycline-inducible OVCAR5 cell line (OVCAR5-tet). OVCAR5-tet cells were infected with Ad.tet.IκBm (10<sup>7</sup>pfu/cell) for 24 hrs, and then added in HEMA-coated culture dishes. These cells grew well on HEMA surface (in absence of adhesion); however, the addition of tetracycline, which induced the expression of dominant negative IκB, resulted in over 80% of cell death in a two-day culture period. In a parallel study, we also tested the sensitivity of ovarian cancer cells in suspension to NF-κB inhibitors, D609 (CalBiochem) and SN50 (BIOMOL). Treatment of OVCAR5 and OVCAR3 with D609 and SN50 induced 46.9 and 33.3% of cell death in first day and 81.2 and 77.2% of cell death in second day, respectively. The results from these studies strongly suggest that Vn/αvβ3 ligation-induced NF-κB activity is essential for ovarian cancer cell survival in suspension.

#### **KEY RESEARCH ACCOMPLISHMENTS:**

- We found that extracellular matrix vitronectin induced NF-κB activity via αvβ3 integrin in human ovarian cancer cells.
- We found that αvβ3 integrin-mediated NF-κB activity is essential for ovarian cancer cell survival in suspension.

#### **REPORTABLE OUTCOME:**

1. Han, Q., New, L., Chen, J., Pan, Z.K., and Huang, S. (2001). Expression of urokinase-specific surface receptor on human carcinoma cells is regulated by interaction with urokinase plasminogen activator. Submitted for *J.Biol.Chem.*

#### **CONCLUSIONS:**

One hallmark of ovarian cancer is the presence of adhering cells on peritoneal surface and floating cells in the ascites. However, it is not clear how ovarian cancer cells can survive in absence of adhesion. Our early studies showed that ovarian cancer cells express both vitronectin and αvβ3 integrin on the cell surface, and the interaction between vitronectin and αvβ3 integrin is essential for ovarian cancer cell survival in suspension. In our recent study, we demonstrated that vitronectin and αvβ3 interaction induced NF-κB activation and this induced NF-κB activity is essential for ovarian cancer cell survival. Our present study may explain why ovarian cancer cells can survive in suspension and implicate a novel strategy for ovarian cancer by disrupting or inhibiting vitronectin/αvβ3 integrin expression.

#### **REFERENCES:**

None

#### **APPENDICES:**

None