AD_____

Award Number: DAMD17-01-1-0383

TITLE: New PTEN Signal Pathway in Breast Cancer

PRINCIPAL INVESTIGATOR: Chawnshang Chang, Ph.D.
Hiroshi Miyamoto, M.D., Ph.D.
Chih-Rong Shyr, Ph.D.

CONTRACTING ORGANIZATION: University of Rochester
Rochester, New York 14627

REPORT DATE: June 2002

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
New PTEN Signal Pathway in Breast Cancer

5. FUNDING NUMBERS
DAMD17-01-1-0383

6. AUTHOR(S)
Chawnshang Chang, Ph.D.
Hiroshi Miyamoto, M.D., Ph.D.
Chih-Rong Shyr, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Rochester
Rochester, New York 14627

E-Mail: chang@URMC.rochester.edu

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. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

Breast cancer is the most common cancer in women, and the second most common cause of cancer death in women in the United States, but the molecular basis remains unclear. PTEN, a tumor suppressor gene is found deleted or mutated in many human tumors, and regulates cell growth, migration, etc through down-regulation of downstream mediators, such as Akt. Overexpression of PTEN in breast cancer cell lines resulted in cell growth suppression. PTEN may be correlated with a positive ER and PR status in primary breast cancers, as well as AR. Androgens, may inhibit mammary carcinoma growth in animal models and is used as a therapeutic agent. Both PTEN and AR play important roles in the progression of breast cancer, however, the correlation between them remains unknown. Based on our preliminary results, we propose a new PTEN pathway by direct interaction with AR results in the modulation of AR-mediated cell growth. Therefore, this study provides a new molecular mechanism of PTEN-mediated AR suppression signaling pathways, which may modulate the cell growth in breast cancer. The consequence of these results may provide new gene therapies or drug designs for treatment of breast cancer patients in the future.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, PTEN, androgen receptor, estrogen receptor	5
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Progress Report. Key Research Accomplishments, Reportable Outcomes, and Conclusions.

Introduction Update. PTEN has been characterized as a tumor suppressor gene and found to be deleted or mutated in a variety of human tumors, including breast cancer. PTEN functions with a dual-specificity protein and lipid phosphatase and negatively regulates cell growth, migration, invasion, and focal adhesions through down-regulation of downstream mediators, such as Akt, focal adhesion kinase, and Shr. Consistent with its tumor suppressor role, overexpression of PTEN in breast cancer cell lines may result in the suppression of the cell growth. Additionally, germline mutations in PTEN are associated with Cowden syndrome (CS), a rare autosomal disease characterized by an increased risk of developing breast cancer. On the other hand, androgen, acting through AR, has been suggested to inhibit mammary carcinoma growth in animal models and has been used clinically as an adjuvant therapeutic agent to influence breast cancer progression. In addition, the germline mutation of AR gene has been shown to cause partial androgen insensitivity together with familial male breast cancer, and the longer CAG repeat (>28) within AR gene (causing lower AR activity) combined with BRCA1 germline mutations is associated with an earlier age of onset of breast cancer development, suggesting that loss or reduction of AR function is implicated in the development of breast cancer. AR has been suggested to be importantly involved in breast cancer initiation. It also has been found that approximately 75%-80% of primary breast tumor specimens tested are positive for AR. Because PTEN expression is correlated with a positive ER and PR status in primary breast tumors and frequently reduced in advanced stage of breast cancer, it is conceivable to propose that PTEN counteracts AR in the mammary tissue to increase the susceptibility to transform to malignancy in the early stage of breast cancer development. Therefore, it will be interested to study the relationship between PTEN and AR in breast cancer and to determine if PTEN may modulate cell growth through AR.

Our studies have involved the MCF-7 breast cancer cell line and results are best summarized in the two figures presented below.

In Figure 1,we demonstrate that AR and PTEN are endogenously expressed in MCF7 cells. And we found that these two proteins can interact with each other in this cell line by co-immunoprecipitation.

As shown in Figure 2, using GST pull-down assay, we found that PTEN and AR are associated directly via the PTP domain of PTEN and AR DNA binding domain.

These studies were reported in a poster format at the Era of Hope Symposium in Orlando, Florida, September 25-28, 2002 and will be submitted along with other results to a peer reviewed Journal in the near future.

Figure 1. PTEN interacts with AR endogenously in the MCF-7 breast cancer cell line.

The MCF-7 cells were cultured in RPMI with 10% Charcoal-dextran treated fetal bovine serum media for 16 hours and treated with ethanol or 10 nM DHT for another 16 hours. Then, cells were harvested and subjected to co-immunoprecipitation with anti-PTEN and anti-HA (control) antibodies, followed by Western Blotting with AR and PTEN antibodies. Results shown are representative of several individual experiments.

Figure 2. GST or GST-PTEN incubation with the [^{35}S]-labeled AR, ER, or RXR for 2 hours in the presence or absence of the ligand. The bound proteins were analyzed by SDS-PAGE, followed by autoradiography. B. Representation of PTEN deleted mutants. PTP domain, protein tyrosine phoshates domain; ty-p, tyrosine phosphorylation domain. C. [^{35}S]-labeled AR was incubated with different PTEN deleted mutants. The nearly equivalent aliquots of PTEN deleted mutants used are shown in the right panel. D. Representation of AR deleted mutants. E. GST or GST-PTEN was incubated with different AR deleted mutants. The SDS-PAGE data represents results from several individual experiments.

Figure 1.

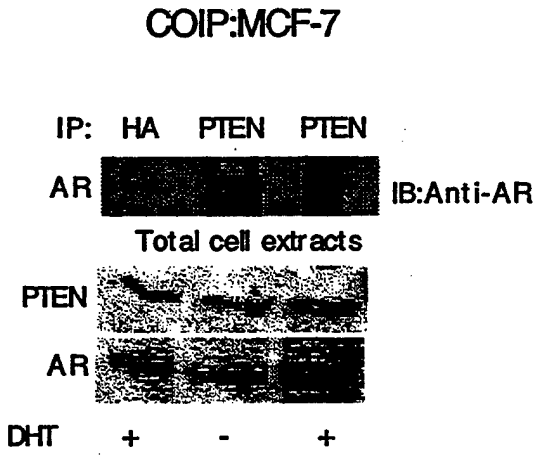

Figure 2.