AD________________

Award Number: DAMD17-99-1-9132

TITLE: Analysis of the TACC1 Gene from the 8p11 Chromosome Region Frequently Amplified in Metastasizing Breast Cancer

PRINCIPAL INVESTIGATOR: John K. Cowell, Ph.D.

CONTRACTING ORGANIZATION: Health Research, Incorporated
Buffalo, New York 14263

REPORT DATE: June 2002

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.

20021024 077

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

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
June 2002

3. REPORT TYPE AND DATES COVERED
Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Analysis of the TACC1 Gene from the 8p11 Chromosome Region Frequently Amplified in Metastasizing Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9132

6. AUTHOR(S)
John K. Cowell, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Health Research, Incorporated
Buffalo, New York 14263

E-Mail: John.Cowell@RoswellPark.org

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)*
The TACC1 gene was isolated from a region of chromosome 8p11 which shows amplification in breast cancers, especially those which show metastasis to the lymph nodes. Preliminary studies showed that overexpression of TACC1 in mouse fibroblasts cause morphological transformation. We have now shown, using immunohistochemistry and immunoprecipitation, that TACC1 is present in the cytoplasm and the nucleus and furthermore that it probably associates with the centrosome and mitotic spindle apparatus during mitosis. Using the full length TACC1 protein as bait to screen a human mammary epithelial cDNA library, we have identified two genes that are also amplified and overexpressed in tumours derived from different cellular origins. TACC1 interacts with the microtubule associated ch-TOG protein, and the oncogenic transcription factor GAS41. TACC1 also associates with L-Sm7, which is involved in RNA splicing and degradation. This suggests that the TACC proteins can form multiple complexes, dysregulation of which may be an important step during progression of breast tumors. Further characterization of the intracellular pathways which use TACC1 for their function will help further understand its role in breast tumorigenesis.

14. SUBJECT TERMS
breast cancer

15. NUMBER OF PAGES
6

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

Cover....................1

SF 298....................2

Table of Contents.................... 3

Introduction....................4

Body....................4-6

Key Research Accomplishments....................6

Reportable Outcomes....................6

Conclusions....................6

Introduction

This is the second year report for this grant which essentially covers a 4 month period from Mar/02 –Jun/02. In November/00 the PI relocated his laboratory from the Cleveland Clinic to Roswell Park Cancer Institute. With delays in providing relinquishing notices from the CCF and delays in effecting the transfer by DOD we have only recently able to assign a replacement scientist to the project.

Body

We previously reported that we raised polyclonal antibodies specific to the TACC1 protein. Using these antibodies we have been able to examine the expression of the TACC1 protein in cell lines available to us. MDA-MB-468 and T47D cells lines showed overexpression of TACC1 in these assays, compared to the immortalized, but not transformed MCF10A cells. Amplification of the 8p11 region has not been observed in these cell lines, suggesting that TACC1 expression may be aberrantly regulated by cell signaling pathways that are disrupted in breast tumors. Intriguingly, MCF7 express TACC1 at relatively low levels compared to MCF10A. The preparation of an antibody and the demonstration of expression of TACC1 in breast tumors cells completes task 1 of the original proposal.

It has been repeatedly demonstrated that DNA amplification results in the overexpression of critical gene(s) within the amplicon, which then results in the increased proliferation of the cancer cell. We have now determined the conditions required to introduce TACC1 constructs into breast tumor cell lines, such as MCF7 and MDA-468, lacking the 8p11 amplicon. We are now in the process of introducing these new constructs into these cells and will generated stable cell lines expressing TACC1. These cell lines will allow us to measure alterations in cellular growth and division rates, to see if either of these constructs lead to a phenotype which mimics the progression to higher grade malignant tumors. Because there is a correlation between 8p11 amplification and metastasis to the axillary lymph nodes, it will also be important to demonstrate whether these constructs will alter the motility of these cells. We will next perform cell migration assays, to show whether breast cancer cell lines expressing these constructs have an increased ability to migrate *in vitro* in a manner analogous to metastasis, and whether there is a correlation between phenotype and the levels of expression of the TACC1 protein. These overexpressing cell lines will also be used to analyze the intereactions between TACC1 and potential binding proteins in the context of breast cancer cells.

To elucidate the potential function of TACC 1, we have chosen to identify proteins that may interact with TACC1. We are using an *in vivo* system, the yeast two-hybrid assay for detecting potential protein-protein interaction by a functional complementation assay in yeast. Functional interactions between the target protein and proteins expressed in frame from the cDNA library of interest are detected by expression of two reporter genes in the yeast genome. Positive clones can then be isolated and sequenced. Coimmunopreciptitation and *in vitro* association techniques are then used to confirm that these interactions occur within mammalian cells.

Our initial search of potential TACC1 interacting proteins used an adult cDNA library, derived from bone marrow. This screen identified two known genes, SIAH1 and

GAS41, two proteins implicated in potential growth control pathways in different cell types. We have now repeated this yeast two hybrid screening procedure, this time using an adult mammary epithelial cDNA library (Clontech). Approximately 10^6 transformants were plated and selected on His^- selective media containing 10mM 3-aminotriazole. Of the 69 His^+ clones originally isolated, 38 proved positive when assayed for β-galactosidase activity using the colony lift assay. Encouragingly, a proportion of clones represented the SIAH1 and GAS41 proteins described above, suggesting that TACC1 could interact with these proteins in normal mammary epithelial cells. In addition, subsequent isolation and sequence analysis revealed that four clones corresponded to the carboxy terminal 529 amino acids of the human ch-TOG protein. ch-TOG is overexpressed in colonic and hepatic tumours, relative to normal tissues, suggesting that the TACC1-chTOG complex could also impact on the dynamics of cell proliferation and transformation. During interphase, the human TACC proteins are found at low levels within the cytoplasm, with an increased accumulation of TACC1 in the nuclei of most cells within the cell population. Upon entering mitosis, the TACC1 begins to associate with the centrosome and the mitotic spindle, although the interaction between TACC1 and microtubules appears to be indirect, requiring the presence of another protein. The ch-TOG protein fulfills this role in that it is able to interact directly with microtubules. Interestingly, the clones that we identified corresponded to the carboxyl terminal 529 amino acids of ch-TOG. This region contains the tubulin dimer-binding domain, required for microtubule nucleation. Thus, by binding to carboxy terminus of ch-TOG, TACC1 could either compete with, or stabilise the binding of the tubulin dimer to ch-TOG. Alternatively, through its interaction with ch-TOG, TACC1 could bring regulatory proteins into the vicinity of the growing microtubule. In either scenario, aberrant TACC1 expression could have a profound effect on the microtubule network and mitotic spindle, leading to cell cycle and mitotic division defects in breast tumor cells.

We have previously shown that native TACC1 shows an enhanced accumulation in the nucleus of cells in culture. Similarly, nuclear accumulation of TACC2 also increases in human microvascular endothelial cells in response to treatment with erythropoietin. This suggests that TACC proteins may play a role in signal transduction to the nucleus in response to certain cytokines. The murine Tacc3 protein, AINT, has recently been shown to interact via the TACC domain with the ARNT transcription factors. Thus it is tempting to speculate that TACC proteins may be involved in the final stages of signal transduction in the nucleus, providing a structural link enhancing the binding of DNA sequence specific transcription factors, such as ARNT, to a GAS41/NuBI1 containing basic transcription factor complex in the nucleus. This hypothesis is further supported by the observation that overexpression of Tacc3 can enhance the ARNT mediated hypoxic induction of the erythropoietin promoter, and can also increase the activation of a xenobiotic response element-luciferase reporter by dioxin. Dysregulation of the TACC1 and GAS41 proteins may therefore contribute to breast tumourigenesis by altering the transcriptional response to cell signaling pathways.

Another protein identified from the mammary epithelial cDNA library is L-Sm7. LSM-7 is a component of a protein complex involved in splicing of pre-mRNAs, as well as their subsequent degradation. We have mapped the precise location of the binding site in TACC1 for this protein by using smaller sections of the TACC1 cDNA and carrying out yeast two-hybrid analysis. The binding site for LSm-7 partially overlaps those of

GAS41 and SIAH1, suggesting that these proteins could compete with each other to bind TACC1. However, LSm-7 also binds to the conserved coiled coil domain, suggesting that these proteins could also interact with the TACC domain of TACC2 and TACC3. Antibodies to LSm-7 do not exist. Therefore, we are currently cloning this cDNA into vectors, which will allow expression of this protein as an EGFP fusion product. When transfected into mammalian cells, we will then be able to verify the interaction between TACC1 and LSm-7 by coimmunoprecipitation. We will then be able to examine how these interactions alter during progression of breast tumors to higher grade metastatic carcinomas.

Thus, we have fully verified the interaction of GAS41, and ch-TOG with TACC1 in breast cancer cells. Verification of interactions with LSm-7 and SIAH1 proteins are still ongoing. Our original task 6 was to screen cDNA libraries to identify full length genes interacting with TACC1. This task has largely been unnecessary to this point since all of the genes showing interactions are available as full length sequences.

Task 1	Complete
Task 2	In Progress
Task 3	Complete
Task 4	Complete
Task 5	In progress
Task 6	Redundant

Key Research Accomplishments

1) Demonstration that TACC1 interactions with the transcription factor GAS41 using immunoprecipitation, and immunohistochemistry
2) Identification of the interaction between TACC1 and ch-TOG, a protein involved in the maintenance of the mictrotubule network
3) Identification of the potential interaction of LSm-7, a protein involved in RNA splicing and degradation.

Reportable Outcomes

Lauffart B, Howell SJ, Tasch JE, Cowell JK, and Still IH. (2002). Interaction of the Transforming Acidic Coiled-coil 1 (TACC1) protein with ch-TOG and GAS41/NuBI1 suggests multiple TACC1 containing protein complexes in human cells. Biochemical Journal 363:195-2002.

Conclusions

We have generated a TACC1-specific polyclonal antibody, which has allowed us to confirm that TACC1 interacts with GAS41, strongly suggesting a role in transcriptional regulation in breast cancer cells. This view is further supported by the immunohistochemical demonstration of an enrichment of TACC1 in the nucleus of interphase cells. Furthermore, during mitosis, TACC1 associates with the mitotic spindle, possibly via the chTOG protein. Attempts are underway to create stable, TACC1 overexpressing cell lines in order to further characterize the role of TACC1 in signal transduction, the cell cycle and mitosis and how this promotes the malignant phenotype.