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Frequently Amplified in Metastasizing Breast Cancer

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

CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation
Cleveland, Ohio 44195

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13. ABSTRACT (Maximum 200 Words) 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 causes 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 mitotic spindle microtubules. Yeast two hybrid analysis has identified a series of genes which interact with TACC1. Two of these, SIAH1 and GAS41, appear to be associated with growth regulatory pathways which may implicate TACC1 in cell growth control and hence why overexpression leads to progression of breast tumors. Two other proteins, p16 and L-Sm7, also interact with TACC1 and these proteins are apparently involved in the transportation of proteins to the nucleus. Thus it appears that TACC1 makes important associations in the cytoplasm which then facilitate the transportation of TACC1 and probably the whole protein complex into the nucleus where it may affect mitosis. Further characterization of the intracellular pathways which use TACC1 for their function will help further understand its role in breast tumorigenesis.					
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INTRODUCTION

The analysis of the 8p11 chromosome region identified a gene, TACC1, which spans 270 Kb of an approximately 350 Kb amplicon frequently seen in breast cancers which show nodal involvement. We had isolated and characterized the full length cDNA and shown that its introduction into NIH cells produced a transformed phenotype. The chromosome location together with the *in vitro* data suggested that TACC1 may play a role in the progression of some breast cancers. In this project we proposed to study the function of this gene and in attempt to understand its potential role in breast tumorigenesis.

BODY

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. Southern blot analysis has now suggested that amplification of 8p11 in breast cancer is associated with rearrangement of the 3' untranslated region (3' UTR) of the *TACC1* gene. We would predict that, as has been noted for cyclin D1, the disruption of the 3' UTR could alter the stability of the TACC1 RNA in these tumours. In our initial assessment of the oncogenic potential of TACC1, we used a construct lacking the majority of the 3' UTR. To determine whether the RNA generated from this construct is more stable than the normal TACC1 RNA, we have now constructed the whole 7kb TACC1 construct, containing the whole of the 3' UTR. 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 generate stable cell lines expressing either the original cDNA, or the full length 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.

Characterizing the function of TACC1 ultimately depends on establishing its intracellular location. According to PSORT predictions, the predicted 88kD TACC1 protein contains two nuclear localization signals, NLS1 and NLS2. This suggested, but did not prove that TACC1 is localized to the nucleus. We have now determined the normal subcellular distribution of the human TACC proteins using EGFP-tagged proteins and by standard immunohistochemical stains using antibodies generated to the TACC proteins (Gergley et al., *in prep.*). During interphase, the TACC proteins are distributed both in the cytoplasm and the nucleus, although antibodies raised to TACC2 strongly stain the centrosomes. In mitotic HeLa and primary fibroblasts, the TACC proteins stain the mitotic spindle and the centrosomes to varying extents. Transient overexpression of the TACC proteins results in the formation of large polymers in the cytoplasm, which retain the ability to bind to microtubules in a regulated manner. This accumulation does not occur in the absence of the TACC domain, suggesting that the TACC proteins interact with microtubules either directly or indirectly through the conserved TACC coiled coil domain. However it is unclear whether the main role of the TACC proteins is in the organization of the microtubule network, or that TACC proteins perform additional functions distinct from their association with microtubules.

To elucidate the potential function of TACC 1, we have chosen to identify proteins which 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.

Coimmunoprecipitation 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. Both of these proteins are expressed in the normal breast tissue. However, because antibodies are not currently available for SIAH1 and GAS41, we have fused these cDNAs to the hemagglutinin and EGFP epitopes. In *in vitro* association studies, we have shown that both recombinant GAS41, and SIAH1 interact with *in vitro* translated, radioactively labeled TACC1. These same constructs will now be introduced into the TACC1 expressing cells described above. The resulting HA-tagged protein should then coimmunoprecipitate with TACC1 using anti-HA or anti-EGFP antibodies. We have determined that EGFP tagged GAS41 shows the same cellular distribution as TACC1. In addition, SIAH1 is localized to both the cytoplasm and the nucleus. The interactions between TACC1, SIAH1 and GAS41 could therefore occur in the cytoplasm and/or the nucleus. Hence we will also prepare extracts from different subcellular compartments, to determine whether the subcellular interaction between TACC1 and these factors is regulated. Because we have shown that overexpression of TACC1 causes aberrant accumulation of TACC1 in the cytoplasm and a potential disruption of the microtubule network, such interactions could also be altered in breast tumor tissues.

Recently a commercial mammary gland cDNA library (Clontech) has become available which has allowed us to identify TACC1 binding proteins in normal breast tissue. Our initial screen of one million cDNA clones from this library identified sixty nine clones by nutrient selection. Encouragingly, a proportion of clones represented the SIAH1 and GAS41 proteins described above, suggesting that these proteins do interact with TACC1 in both normal breast tissue, and potentially in breast tumors. The remaining clones are currently being isolated and sequenced. The first series of clones isolated correspond to two recently identified proteins. The first protein identified, is the human orthologue of p16, a bovine protein involved in transport of proteins from the endoplasmic reticulum to the golgi apparatus prior to sorting to different subcellular compartments. The second protein identified, L-Sm7 is a component of a protein complex assembled in the cytoplasm and transported to the nucleus. This complex is a key component in splicing of pre-mRNAs. We have mapped the precise location of the binding site in TACC1 for these proteins by using smaller sections of the TACC1 cDNA and carrying out yeast two-hybrid analysis. The binding site for p16, and LSm-7 partially overlap those of GAS41 and SIAH1, suggesting that these proteins could compete with each other to bind TACC1. However, p16 and LSm-7 also bind to the conserved coiled coil domain, suggesting that these proteins could also interact with the TACC domain of TACC2 and TACC3. Antibodies to p16 and LSm-7 do not exist, therefore, we are currently cloning these cDNAs into vectors, which will allow expression of these proteins as EGFP fusion products. When transfected into mammalian cells, we will then be able to verify the interaction between TACC1 and these proteins by coimmunoprecipitation. Furthermore, we will assess whether these proteins interact with the other members of the TACC family. We will then be able to examine how these interactions alter during progression of breast tumors to higher grade metastatic carcinomas.

KEY RESEARCH ACCOMPLISHMENTS

1. 8p11 amplicons disrupt the 3' untranslated region of the TACC1 gene
2. TACC proteins are distributed throughout the cell, and are associated, either directly or indirectly with the microtubule network
3. Identification of TACC1 binding factors from the mammary gland. GAS4, SIAH1, and two new binding factors, p16 and LSm-7

REPORTABLE OUTCOMES

The TACC domain proteins are concentrated at centrosomes and can form large polymers that interact with microtubules. Fanni Gergely, Christina Karlsson, Ivan Still, John Cowell, John Kilmartin, and Jordan Raff (in preparation)

CONCLUSIONS

We have used immunohistochemical approaches to demonstrate that the TACC1 gene product is present in both the cytoplasm and the nucleus and we have strong evidence that it associates with the microtubules. We have used the yeast 2-hybrid system to isolate several genes which interact with TACC1. Two of these genes are implicated in growth control pathways and so support the idea that TACC1 may be involved in tumor progression. The observation that TACC1 also associates with proteins which are involved in trafficking to the nucleus suggests that TACC1 is either mediating the transfer of protein complexes to the nucleus or is using these complexes to gain access to the nucleus itself, where it potentially affects cell growth. Further elucidation of the function of TACC1 will allow us to establish its role more fully

REFERENCES

None