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13. ABSTRACT *(Maximum 200 Words)*

Breast cancer arises from a series of harmful mutations to genes important for the normal regulation of cell growth and differentiation. Identification of the gene products whose loss is important in the development of the cancer is the primary means of determining who is at risk. There is an acute need for a more comprehensive understanding of the gene products that contribute to regulation, and the consequences of their failures. Gene products implicated in estrogen-responsive pathways are particularly likely to be significant in tumorigenesis because exposure to estrogen is one of the most important contributory factors for the development of breast cancer. Our lab has cloned a new gene, p270, which codes for a protein that has structural characteristics and biochemical properties suggesting that it plays a significant role in the regulation of gene expression in response to estrogen. This project is designed to test this possibility by analyzing p270 expression and function in normal and breast cancer cells. This analysis will advance our understanding of the molecular mechanisms underlying normal breast development and carcinogenesis. These studies are likely to identify new markers for diagnosis and prognosis. They may ultimately lead to the design of therapeutic strategies based on the function of p270. These studies are particularly likely to open up new perspectives and stimulate new initiatives in the search for a cure for breast cancer.

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Introduction

p270 was first identified in this lab during studies of proteins, such as p300 and the viral oncogene E1A, that affect gene expression during growth and differentiation. The structural similarity of p270 and p300 led to the isolation and cloning of p270 (Dallas *et al.*, 1997; 1998; 2000). Analysis of p270 associated proteins visualized in immune complexes showed that p270 is an integral member of human SWI/SNF complexes (Dallas *et al.*, 1998). Several months ago, identification of a band designated BAF250 in an hSWI/SNF complex defined by association with the SWI2-related protein, BRG1, was reported. In agreement with our results, sequencing showed that this band is indeed p270 (Nie et al., 2000). SWI/SNF complexes were first discovered in yeast cells where they are involved in the regulation of genes that are required for yeast growth and mating type switch (reviewed in Peterson and Workman, 2000). Isolation of *Drosophila* and human homologues of yeast SWI/SNF complex components suggests that SWI/SNF complexes play fundamental roles in the regulation of gene expression during growth and development in all eukaryotic organisms (reviewed by Kingston et al., 1996; Kadonaga, 1998).

Cloning of p270 in this lab (Dallas et al., 1998) revealed that p270 belongs to a newly identified ARID DNA binding protein family. Members of this family play important roles during normal development and tissue specific gene expression (reviewed in Kortschak et al. 2000). Besides the ARID consensus, p270 possesses multiple signature nuclear hormone receptor interaction motifs, most of which are clustered in its C-terminal part. (Dallas *et al.*, 1998, 2000; Nie et al., 2000). These properties give p270 the potential to mediate gene responses to hormone signals.

Body

My application had three objectives:

1. To determine whether expression of p270 influences transcriptional activation from estrogen responsive promoters in transient transfection assays.
2. To determine whether p270 expression is altered in hormone dependent and independent breast cancer lines.
3. To determine whether p270 is required for growth regulation by human SWI/SNF complexes

I originally planned to address all aims simultaneously. However, the Peer Review Panel Report cautioned very strongly that my proposal was overly ambitious. Therefore, I decided to put major focus on one aim at a time. Most of my work has focused on aim 2, for its direct emphasis on breast cancer.

I originally proposed to use the monoclonal antibody (mAb) NM1 to screen for p270 expression and the composition of hSWI/SNF complexes in a panel of breast cancer lines. The NM1 mAb was raised against p300 and recognizes an epitope also present on p270. However, my lab has since characterized a protein closely related to p270 which can also associate with SWI/SNF complexes, and which is also seen by NM1. (This protein is presently designated pKIAA1235). To understand the role of p270 in hSWI/SNF complexes in breast cells, it is therefore essential to have an antibody specific for p270.

When I began my project our lab already had rabbit antipeptide antibodies specific for an epitope near the C-terminus of p270. This antibody, like the majority of anti-peptide antibodies, recognizes denatured protein in procedures such as Western Blots, but is not capable of immunoprecipitating native p270. To facilitate further study of p270, we needed to raise antibodies suitable for precipitation of p270-specific immune complexes. Mice were immunized with a recombinant p270 fragment containing the DNA binding region and surrounding sequences. The rationale for choosing this portion of p270 for antibody generation was that the DNA binding region was likely to be on the surface of the native protein. In addition, this region was least likely to be the site of protein interactions, so that antibodies raised against this region would hopefully not compete with other protein interactions on the surface of native p270. Three hybridoma lines were generated against this fragment. They are designated PSG1, PSG2, and PSG3. PSG3 is the best, and one which I have selected for fuller characterization. I have determined that PSG3 recognizes p270 in immune precipitations from native cell lysates. This antibody also

recognizes denatured proteins tested by Western blotting. Importantly, PSG3 does not recognize the closely related KIAA1235 protein. Using PSG3 I also determined by immunofluorescent staining that p270 is mainly a nuclear protein.

A close study of the immune complex precipitaed by the new PSG3 antibodies revealed that it comprises the whole SWI/SNF complex plus seven or more unknown proteins. I determined by depletion of SWI/SNF complexes using an antibody to the BAF155 complx component, that p270 is present in cells in excess of the SWI/SNF complexes, such that separate populations of p270 are associated with the SWI/SNF complexes, and the unidentified series of proteins (**Fig. 1**). These observations indicate that p270 may be involved in other important cellular associations during the regulation of cell growth and gene expression. Screening of multiple cell lines with PSG3 indicates that the same p270 associations exist in all human and mice lines tested, including the breast cancer line MCF7. The associated proteins include several highly phosphorylated species, leading me to suspect that a kinase activity might be present. I have demonstrated that there is, indeed, a p270-associated kinase activity, and that it is associated with the population of p270 that is distinct from the SWI/SNF complexes. Because the loss of p270 during carcinogenesis will affect the functions of these unknown proteins as well as the SWI/SNF complexes, I plan to identify these proteins as part of this proposal. This goal was not included in my original statement of work - but any attempt to understand the loss of p270 expression in breast cancer cells, must take into account the significance of these associations as well as the SWI/SNF complex associations. I have done pilot experiments to determine that I can feasibly obtain a yield of the novel p270-associated proteins sufficient for mass spectrometric analysis. Approximately 180 mg total cell lysate yields sufficient protein for sequencing of at least four protein species. Sequencing will be done at the Harvard Microchemistry Facility.

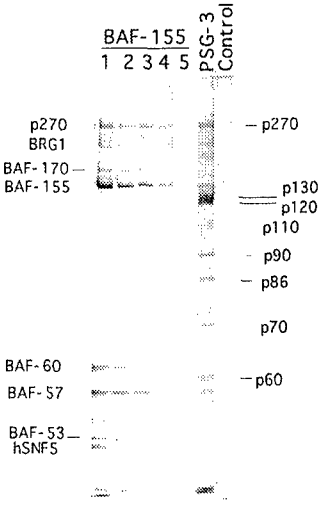

Fig. 1. p270-associated proteins include a series of novel species in addition to hSWI/SNF complex components. ^{35}S-labeled cell lysates were subjected to 5 successive immunoprecipitations with BAF155 mAbs. This reaches the limit of the ability of this antibody to bring down BAF155 and the associated hSWI/SNF complex components (identified at the left of the figure). However, p270 is not depleted, and can still be immunoprecipitated with the PSG3 mAbs, along with a series of novel p270-associated proteins (indicated to the right of the figure).

With the newly characterized antibody PSG3, I began a screen of breast cancer cell lines by Western Blot. Five lines are shown in **Fig.2**, along with cervical carcinoma lines HeLa and C33A, as well as normal human embryo lung fibroblasts WI-38, the osteosarcoma line SAOS-2, and a prostate cell line PC-3. I have also determined that p270 expression levels are normal in MCF7 cells. At the time of my proposal, I already knew p270 was undectable in C33A cells. I predicted that p270 expression would be abnormal in a subset of breast cancer lines as well. The screen now shows that this prediction was accurate as p270 expression is undetectable in T47D breast carcinoma cells.

While this work was in progress, a report appeared on expression of human SWI/SNF complex components in tumor cell lines originating from various types of tumors (DeCristofaro et al., 2001). This study scored 2 of 21 breast cancer cell lines as reduced for p270 expression. This preliminary study further supports our prediction that p270 expression and/or function is altered in breast cancer cell lines. These authors suggest that p270 is destabilized at the protein level. However, I have begun to probe the basis for altered p270 expression, first in C33A cells, and find that these cells have very low levels of p270 RNA (**Fig. 3**), suggesting a very different mechanism. p270 is more likely to be down-regulated at the level of gene expression - or possibly even due to bi-allelic inactivation. I am currently extending the RNA studies to the p270-negative breast cancer lines.

As discussed in my proposal, the presence of normal levels of p270 in the majority of breast cancer lines does not mean that the p270 population is functional. the confirmation of our prediction that loss of p270 would be apparent in some cell lines supports my hypothesis that p270 function may be altered in lines where expression appears normal. In the past year, I have set up the p270 functional assays discussed in my application. I will screen the panel of breast cancer cell lines for the integrity of the SWI/SNF complex. It is possible that p270 may be present in these lines, but no longer able to associate with the complex. I have also set up assays to determine whether p270 retains the ability to bind native DNA, and to measure the p270- associated kinase and ATPase activities.

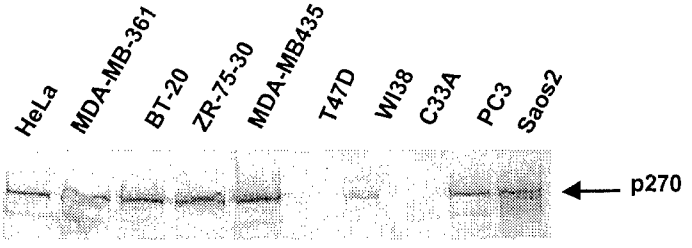

Fig. 2. Western Blot analysis of p270 in total cell lysates of breast cancer lines. 150 microgram aliquots of each lysate were separated by SDS-PAGE, transferred to PVDF membrane, and probed with monoclonal antibodies PSG3 specific for p270.

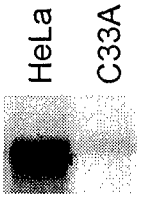

Fig. 3. p270 expression is reduced at the RNA level in the cervical carcinoma cell line, C33A. 20 µg total cell RNA isolated from HeLa or C33A cells was electrophoretically separated and transferred to nitrocellulose. The blot was hybridized with a probe representing p270 cDNA sequences. Quantification by phosphoimager indicates that the expression level in C33A cells is about 17% of the level in HeLa cells.

Key Research Accomplishments

- Characterized a new series of monoclonal antibodies, specific for p270 and capable of immunoprecipitating p270 and the associated hSWI/SNF complex from all cell lines tested, including MCF7 breast cancer cells.
- Demonstrated that p270 is present in cells in excess of the SWI/SNF complexes. Separate populations of p270 are associated with the SWI/SNF complexes, and with an unidentified series of at least six proteins.
- Demonstrated that there is a p270-associated kinase activity, and that it is associated with the population of p270 that is distinct from the SWI/SNF complexes.
- Screened a panel of breast cancer cell lines and found that p270 expression is lacking in T47D cells.
- Reported my results on the p270-specific monoclonal antibodies and the newly revealed p270-associated proteins at the Small DNA Tumor Viruses and Cell Cycle Control Meeting, held at the University of Wisconsin, Madison in July, 2000. My abstract was selected for oral presentation, and for a competitive travel award.
- Presented my work on the p270-specific monoclonal antibodies and the newly revealed p270-associated proteins at the annual Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Student/Postdoctoral Associate Research Day, held on December 01, 2000. This research competition is judged by prominent scientists from outside of Temple University. My presentation was selected as First Prize Winner among all student and postdoctoral presentations submited.

Reportable Outcomes

Abstracts:

Xiaomei Wang, Deborah Wilsker, Stephen Pacchione, Peter Yaciuk, Peter B. Dallas, and Elizabeth Moran. *The Human SWI-SNF Complex Protein p270 Exists in a Novel Cellular Complex that Has a Kinase Activity*. Selected for oral presentation at the 2000 Small DNA Tumor Viruses Meeting, University of Wisconsin, Madison.

Deborah Wilsker, Antonia Patsialou, Norm Nagl, Xiaomei Wang, Michael Van Scoy, Peter B. Dallas, Takahiro Nagase, and Elizabeth Moran. *Analysis of Structure and Function Relationship in p270, a Human SWI/SNF Complex Protein.* Selected for oral presentation at the 2000 Small DNA Tumor Viruses Meeting, University of Wisconsin, Madison.

Xiaomei Wang, Deborah Wilsker, Stephen Pacchione, Peter Yaciuk, Peter B. Dallas, and Elizabeth Moran. *The Human SWI-SNF Complex Protein p270 Exists in a Novel Cellular Complex that Has a Kinase Activity.* Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Student Research Day, December 01, 2000. (Selected as First Prize Winner).

Norman G. Nagl, Jr., Xiaomei Wang, Deborah Wilsker, Michael Van Scoy, Takahiro Nagase, Peter Dallas , Peter Yaciuk , and Elizabeth Moran. *Characterization of a Novel Paraloque of Human SWI/SNF Member p270.* To be presented at the 2001 Meeting on Small DNA Tumor Viruses and Cell Cycle Control, Cambridge University, Cambridge, UK.

Papers in Preparation:

Xiaomei Wang, Stephen Pacchione, Peter Yaciuk, Peter B. Dallas, and Elizabeth Moran. Generation of monoclonal antibodies specific for p270.

Xiaomei Wang, Da-Wei Laio, Stephen Pacchione, Peter Yaciuk, Peter B. Dallas, and Elizabeth Moran. Generation of monoclonal antibodies specific for the BAF155 component of hSWI/SNF complexes.

Xiaomei Wang, Peter B. Dallas, and Elizabeth Moran. p270 is down-regulated at the protein and RNA level in human tumor cell lines.

Conclusions

I have characterized p270-specific antibodies and successfully begun to screen breast cancer cell lines for aberrant p270 expresion. The new antibodies have also revealed the presence of additional p270-associated proteins, distinct from members of the SWI/SNF complex. I am continuing to probe the panel of breast cancer lines for alterations in p270 function. I will probe biochemical activities associated with p270, as well as the integrity of the SWI/SNF complex. I also plan to identify the p270-associated proteins that are not in the hSWI/SNF complex. Given that p270 expression is altered in breat cancer lines, as predicted, an understanding of the consequences of loss of p270 expression will necessarily include a knowledge of these p270-associated species.

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Appendices: None