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12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
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). 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 indicates 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; Wilsker *et al.*, 2002). 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.

Several lines of evidence suggest that SWI/SNF complexes are required for regulation of cell growth. The core ATPase enzyme of the complex, BRG1, is missing or mutated with high frequency in certain kinds of tumor cells (Muchardt and Yaniv, 1993; Wong, *et al.*, 2000; DeCristofaro *et al.*, 2001). Inactivation of BRG1 in mice showed that it is indispensable for viability. BRG1 heterozygous mice are predisposed to tumors (Bultman *et al.*, 2000). Restoration of BRG1 expression in BRG1-deficient cells is sufficient to reverse the transformed phenotype (Wong *et al.*, 2000). Inactivation of other complex components is also linked with tumorigenesis. For example, deficiency of hSNF5, another core SWI/SNF component, is directly linked to a malignant pediatric tumor rhabdoid sarcoma (Versteene *et al.*, 1998). Mice with SNF5 heterozygous mutations also developed tumors (Klochendler-Yeivin *et al.*, 2000). This evidence strongly suggests that inactivation of SWI/SNF components is an important process during tumorigenesis. As a core component of SWI/SNF complexes, p270 may act in the same pathway as BRG1 and thus may be required for the tumor suppressor activity of the complex, especially in cells that are responsive to hormones.

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 cautioned very strongly that my proposal was overly ambitious. Therefore, I decided to focus mainly on aim 2, which has direct emphasis on breast cancer.

As reported last year, since the start of this project my lab has characterized a new protein closely related to p270 that can also associate with SWI/SNF complexes. Therefore it is important to have specific reagents to distinguish these proteins. To facilitate the screening for p270 expression in breast cancer cell lines, our lab developed p270-specific mouse monoclonal antibodies. These antibodies are designated as PSG1, PSG2, and PSG3. I have fully characterized PSG3 and determined that PSG3 recognizes both native p270 from cell lysates and denatured p270 immobilized on PVDF membrane. Importantly, PSG3 does not recognize the p270-related protein pKIAA1235, making it possible to obtain a very specific profile of p270 expression in tumor cell lines.

In order to characterize the SWI/SNF sub-unit compositions in general, our lab also developed mouse monoclonal antibodies against other core SWI/SNF components BRG1 and BAF155. With the aid of these antibodies and a commercially available antibody against hBRM, I began to screen a panel of breast cancer cell lines by Western Blot. **Fig.1** shows a sample of five breast cancer cell lines along with the cervical carcinoma lines HeLa and C33A, as well as a normal human embryo lung fibroblasts WI38. My work, combined with emerging data from another lab (DeCristofaro *et al.*, 2001) gives a profile of 24 different breast tumor lines. These studies together give us a good overview of p270 and SWI/SNF expression in breast cancer cell lines. Among the 24 lines screened, 9 lines have at least one other SWI/SNF component missing. These components include BRG1, hBRM, BAF155, BAF57, and hSNF5. 2 lines of out the 24 have altered p270 expression. These data support our prediction that p270 is targeted during tumorigenesis in human breast tissue.

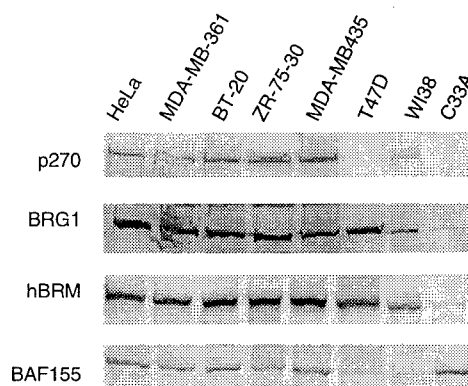


Fig. 1. Western Blot analysis of p270 and other SWI/SNF component in total cell lysate of breast cancer cell lines. 150 microgram aliquots of each lysate were separated by SDS-PAGE, transferred to PVDF membrane, and probed with monoclonal antibodies specific for p270, BRG1, hBRM, and BAF155.

We knew from previous work that p270 is absent in C33A cells. Last year I reported an examination of the level of p270 loss in these cells, showing that p270 is reduced at the RNA level. During the past year, I extended this RNA analysis to T47D cells. The results shown in **Fig.2** revealed that the p270 transcript is not decreased in T47 D cells as it is in C33A. This suggests that very different mechanisms may underlie the loss of p270 in these two cell lines. p270 expression could be down regulated in T47D cells due to protein stability, possibly resulting from amino acid substitutions. In C33A cells, p270 is more likely to be down regulated at the level of gene expression, or possibly due to bi-allelic inactivation. I am continuing to explore the mechanism of p270 inactivation in these cells.

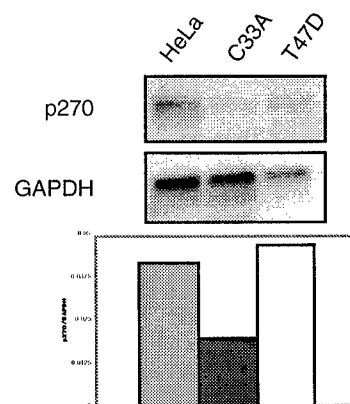
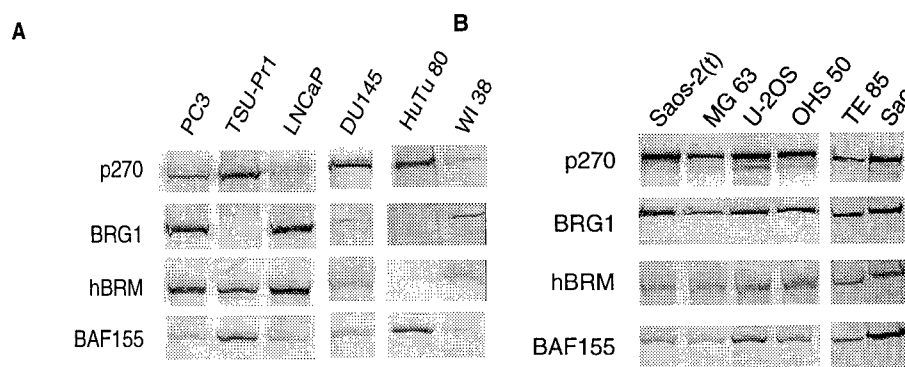


Fig. 2. Northern Blot analysis of p270 mRNA expression in p270 defect cervical carcinoma C33A and breast carcinoma T47D cells. 18µg of poly A RNA isolated from HeLa, C33A, and T47D cells was electrophoretically separated and transferred to Nylon membrane. The blot was hybridized with a probe representing p270 cDNA sequences. p270 signals were quantified and normalized to those of GAPDH by phosphor-imager.

Considering the high frequency in alteration of SWI/SNF components in breast cancer cells, I thought it also important to study expression profiles of p270 and other SWI/SNF components in tumor lines other than breast origin for comparison. **Fig. 3A** shows Western blot analysis of 4 commonly used prostate carcinoma lines and one duodenum carcinoma cell line HuTu 80. BRG1 expression is deficient in DU145 and TSU-Pr-1 lines as reported previously (Wong *et al.*;2000). Western analysis against p270 did not reveal significant change in p270 expression. My analysis did identify a previously unrecognized tumor line in which both of the SWI2/SNF2 family ATPases, BRG1 and hBRM, are missing. This is the duodenum carcinoma line HuTu 80.

In order to have a good view of tumor cells in general, I also extended my screening to a panel of sarcoma cell lines. In contrast to the breast, prostate, and other carcinoma cells, in half of which expression of at least one of the core SWI/SNF proteins examined here was found to be changed, all six osteosarcoma cells I screened have normal levels of p270, BRG1, hBRM, and BAF155 expression (**Fig. 3B**). I am exploring the possibility that a novel SWISNF component may contribute to tumorigenesis in these cells.

Fig. 3. Western Blot analysis of p270 and other major SWI/SNF components in 4 prostate carcinoma cell lines, a duodenum carcinoma line HuTu 80, and 6 osteosarcoma lines. 150 µg of each lysate were separated by SDS-PAGE and transferred to PVDF membranes. The blots were probed with mouse monoclonal antibody specific for p270, BRG1, hBRM, and BAF155.



Last year I reported that with the newly characterized p270 monoclonal antibody PSG3 I revealed that p270 may be involved in other cellular associations during the regulation of cell growth and gene expression. Moreover, the novel associations contain a kinase activity. In the current year I have purified six individual novel p270 associated proteins and characterized them by Mass-spec analysis in collaboration with the Harvard Microsequencing facility. Among them, I identified TRAP150 (Thyroid Receptor Associated Protein 150) as a novel p270 associated protein suggesting further links between p270 and nuclear hormone responsive genes. I also identified a kinase designated AAK (abl Associated Kinase) that may contribute to the kinase activity I observed in the complex. I am now exploring the substrates for this kinase and the significance of these associations.

Key Research Accomplishments

- Screened a panel of breast cancer cell lines and found p270 expression is lacking in T47D cells. Northern blot analysis revealed that a different mechanism may underlie the loss of p270 in this breast cancer line compared with another p270 defect line C33A.
- Extended my screening of p270 to a panel of prostate carcinoma lines as well as osteosarcoma lines.
- Analyzed other major SWI/SNF components in all breast, prostate, and other carcinoma lines, as well as osteosarcoma lines, and found besides p270, expression of other SWI/SNF proteins are frequently changed in the carcinoma lines, but not in the sarcoma lines.
- Identified a potential association between p270 and TRAP complexes, which are mediators of thyroid hormone responsive gene activation. Also demonstrated that AAK is the kinase that associates with the population of p270 that is distinct from the SWI/SNF complexes.
- Reported my work on characterization of p270 and other human SWI/SNF proteins in tumor cell lines at the 93rd AACR meeting held in San Francisco in April 2002.

Reportable Outcomes

Abstracts:

Xiaomei Wang¹, Norman G. Nagl, Jr.¹, Peter Yaciuk², Marc F. Hansen³, Peter B. Dallas⁴, and Elizabeth Moran¹. Characterization of p270 (*SMARCF1*) and other SWI/SNF proteins in human breast and other cancer cell lines. Era of Hope 2002 DoD Breast Cancer Research Program Meeting, September 2002, Orlando, Florida.

Xiaomei Wang¹, Norman G. Nagl, Jr.¹, Peter Yaciuk², Marc F. Hansen³, Peter B. Dallas⁴, and Elizabeth Moran¹. Characterization of p270 (*SMARCF1*) and other human SWI/SNF proteins in tumor cell lines. The 93rd AACR, April 2002, San Francisco, California.

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 Paralogue 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 expression of p270 and other core SWI/SNF components in a panel of humane malignant tumor cell lines. These cell lines include 5 breast carcinoma lines, 4 prostate carcinoma lines, 1 duodenum carcinoma line, and 6 osteosarcoma lines. Together with recently reported study, we observed that p270 and other SWI/SNF components are frequently targeted during tumor formation. Out of the 24 breast cancer cell lines screened by me and different groups, 2 are found with altered expression of p270 while 9 are found with changed expression of one or more other SWI/SNF components. By RNA analysis I observed that loss of p270 expression in tumor cells can be at either protein level or RNA level. I will continue to analyze how p270 contribute to the growth regulations in T47D and C33A cells. I will overexpress p270 in these two p270 deficient cell lines and see whether restored p270 is sufficient to change the transformed phenotype with or without the present of BRG1. I will also evaluate a series of growth phenotypes in these cells, including rate of cell division and level of cyclin-dependent kinase activity.

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