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13. ABSTRACT (Maximum 200 Words)

Breast cancers differ from benign breast epithelium through the prominent expression of pp32r1 and pp32r2, oncogenic members of the pp32 gene family, in contrast to benign epithelium, which predominantly expresses pp32. The purpose of the study is to confirm and extend these preliminary results, to develop practical means to assay pp32 gene family members in clinical samples, and to determine the clinical significance of their presence. The approved proposal encompassed four broad tasks: [1] characterization of the pp32 expression phenotype of a larger sample of 40 prostatic adenocarcinomas; [2] development of a practical molecular pathology assay for altered pp32 transcripts; [3] adaptation of the assay to paraffin-embedded tissue; and [4] preliminary determination of the clinical utility of pp32r1 and pp32r2 expression in prostatic adenocarcinoma. In the course of pursuing this work, we recognized that improved assay methods would yield better results for Task 1. To complete these tasks, it was ultimately necessary to raise, affinity-purify, and characterize antibodies to peptides derived from pp32, pp32r1, and pp32r2. Characterization of these antibodies by Western blot shows that they are sufficiently specific to be employed in panels to distinguish the pp32 forms in breast samples; this work will continue beyond the project period.

14. SUBJECT TERMS

Pathobiology; molecular pathology; translational research; tumor suppressor, pre-invasive breast disease, DCIS, prognosis

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Table of Contents

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

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

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

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

Body....................4

Key Research Accomplishments....................8

Reportable Outcomes....................8

Conclusions....................8

References....................8

Appendices....................8

INTRODUCTION

Since the last report, further progress in the fundamental biology of pp32 has underscored its prominence as a key regulator of important cellular processes:

Reduction of pp32 by antisense or siRNA induces cellular differentiation with discrete associated changes in gene expression profiles (1)

pp32 is a key participant in granzyme A-mediated apoptotic pathways (2,3); it also participates in a mitochondrial pathway regulating caspase activation (4).

pp32 regulates gene expression as part of a complex that inhibits histone acetylation (5)

pp32 is a regulator of mRNA stability and trafficking (6-8)

Taken together, these findings strongly indicate that pp32 is involved in control of the critical programmatic decision that cells make as to whether to retain the capacity to proliferate, or whether to undergo differentiation.

Regarding the specific project funded by USAMRMC, our previous work demonstrated that breast cancers differ from benign breast epithelium in their expression of oncogenic members of the pp32 gene family (9,10). Whereas benign breast epithelium solely expresses pp32, a tumor suppressor, breast cancers express pp32r1 and pp32r2, which are oncogenic. The approved proposal encompassed four technical objectives: [1] characterization of the pp32 expression phenotype of a larger sample of 40 breast cancers; [2] development of a practical assay for altered pp32 transcripts in archival tissue; [3] determination of the association of specific pp32 variants in laser capture microdissected DCIS with invasion and with high-grade comedo DCIS; and [4] application of the results in a retrospective study (if [3] shows meaningful correlations) to a population of patients with follow-up. Since submission and approval of the original proposal, several technical and scientific events have occurred that have positively impacted the strategy, efficiency with which the remaining aims can be accomplished, and dramatically increased the translational potential of the technology should the study establish analysis of the pp32 gene family as a clinically important assay of biologic potential in pre-invasive breast neoplasia.

BODY

Task 1. This task involves characterization of abnormal pp32 transcripts in frozen samples of human duct carcinoma compared to paired normal breast controls. Work under this approved task will entail the analysis of 40 frozen breast cancer specimens by RT-PCR, cloning, and sequencing of 10 to 20 clones of pp32 gene family members derived from the tumors to

detect variant sequences. Additional features of the task involve analysis of the frequency and position of previously uncharacterized changes.

Progress: Investigations of pp32r1 yielded several polymorphisms along with a functional mutation that promoted the growth of a test prostatic adenocarcinoma cell line. The functional mutation was investigated in a panel of human breast cancer cell lines (MCF-7, SKBr3, MDA-435, and ZR-75), but was not found to be present. Evaluation of 24 breast cancer samples representing a cross-section of stages and grades also failed to reveal the mutation.

Task 2. This task involves development of a specific molecular assay compatible with use on archival tissue. The task approved in the proposal involved dissection or laser capture microdissection of tissue, RT-PCR amplification of pp32 gene family members, development of a restriction fragment length polymorphism analysis to distinguish pp32 gene family members from one another, and assay validation.

Progress: Molecular approaches proved too cumbersome for practical use in evaluation of breast cancer specimens. Consequently, polyclonal antibodies to peptides putatively specific for each of the three isoforms were raised collaboratively, affinity-purified, and evaluated by Western blot against GST-fusions of each of the proteins. The results are shown in the figure below:

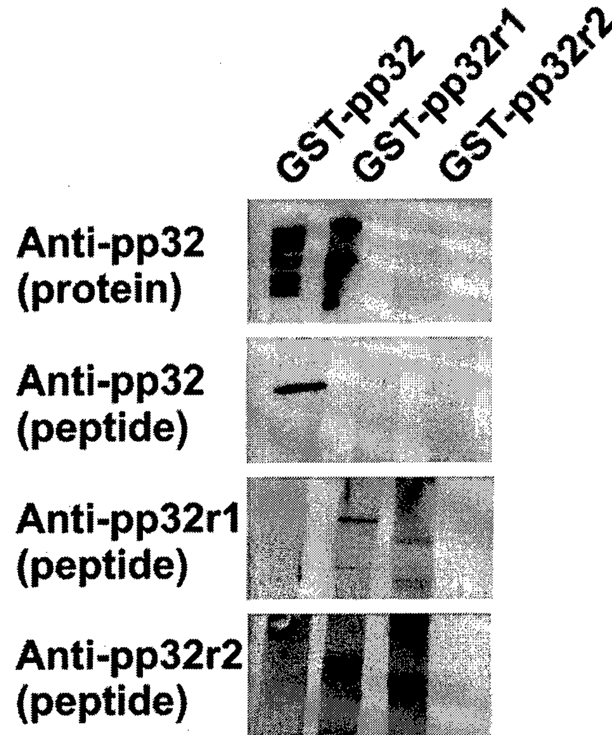

Figure 1. Evaluation of Antibodies to pp32 Gene Family Members

While only one of the antibodies, that to pp32, demonstrated absolute specificity, Table 1, which summarizes the results, shows that the four antibodies are useable as a panel. Each protein, pp32, pp32r1, and pp32r2, yields a different pattern of reactivity that permits definitive identification. Thus, these antibodies can be used to perform the originally intended studies with the absolute confidence that eluded prior attempts at metholdologic development.

Table 1. Summary of Antibody Reactivities

	Antibody			
Protein	anti-pp32 GRP	anti-pp32 MARL	anti-pp32r1 MARL	anti-pp32r2 MARL
pp32	+	+	-	-
pp32r1	+	-	+	+
pp32r2	-	-	+	+

Task 3. Identification of pp32 variants preferentially expressed in DCIS with co-existing invasive duct carcinoma or in high-grade comedo DCIS. This task

involves a comparison of the expression of pp32 variants in pre-invasive breast cancer using the presence of co-existing invasive cancer as a surrogate marker for increased risk. Studies will use the method developed in Task 2 to analyze tissue provided by Dr. David Page, with subsequent statistical analysis of the results for meaningful associations.

Progress: Now that Task 2 is finally complete, work on this task using immunohistochemistry with the antibodies described under Task 2. Work will continue on this project beyond the project period.

Task 4. Determination of the significance of variant pp32 expression as a risk factor for subsequent development of invasive breast cancer (contingent upon Task 3 results).

Progress: Work on this task will commence upon completion of Task 3.

KEY RESEARCH ACCOMPLISHMENTS

After some difficulty caused by unanticipated quantitative differences in pp32, pp32r1, and pp32r2 expression, and by technical difficulties associated with *in situ* hybridization using oligonucleotide probes, antibodies have been developed that can now be applied to the original tasks associated with this project using routine immunohistochemistry techniques.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Antibodies have been developed that reliably distinguish the members of the pp32 gene family. These antibodies will now be applied to the study of human breast cancer in work that will be carried on beyond the funding period in order to complete the tasks.

REFERENCES

1. Brody, J. R., Kadkol, S. S., Hauer, M. C., Rajaii, F., Lee, J., and Pasternack, G. R. (2004) *American Journal of Pathology.* **164**, 273-283
2. Fan, Z., Beresford, P. J., Oh, D. Y., Zhang, D., and Lieberman, J. (2003) *Cell.* **112**, 659-672
3. Fan, Z., Beresford, P. J., Zhang, D., and Lieberman, J. (2002) *Molecular & Cellular Biology.* **22**, 2810-2820
4. Jiang, X., Kim, H. E., Shu, H., Zhao, Y., Zhang, H., Kofron, J., Donnelly, J., Burns, D., Ng, S. C., Rosenberg, S., and Wang, X. (2003) *Science.* **299**, 223-226
5. Seo, S. B., Macfarlan, T., McNamara, P., Hong, R., Mukai, Y., Heo, S., and Chakravarti, D. (2002) *Journal of Biological Chemistry.* **277**, 14005-14010
6. Gallouzi, I. E., Brennan, C. M., and Steitz, J. A. (2001) *Rna.* **7**, 1348-1361
7. Brennan, C. M., Gallouzi, I. E., and Steitz, J. A. (2000) *Journal of Cell Biology.* **151**, 1-14
8. Gallouzi, I. E., and Steitz, J. A. (2001) *Science.* **294**, 1895-1901
9. Kadkol, S. S., El Naga, G. A., Brody, J. R., Bai, J., Gusev, Y., Dooley, W. C., and Pasternack, G. R. (2001) *Breast Cancer Research & Treatment.* **68**, 65-73
10. Brody, J. R., Kadkol, S. S., Mahmoud, M. A., Rebel, J. M., and Pasternack, G. R. (1999) *Journal of Biological Chemistry.* **274**, 20053-20055

APPENDICES

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