

AD \_\_\_\_\_

Award Number: DAMD17-98-1-8070

TITLE: Human Progesterone A-Form as a Target for New Drug  
Discovery in Human Breast Cancer

PRINCIPAL INVESTIGATOR: James W. Voltz  
D. McDonnell, Ph.D.

CONTRACTING ORGANIZATION: Duke University Medical Center  
Durham, North Carolina 27710

REPORT DATE: July 2002

TYPE OF REPORT: Annual Summary

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.

20020606 041

# 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

<b>1. AGENCY USE ONLY (Leave blank)</b>	<b>2. REPORT DATE</b> July 2002	<b>3. REPORT TYPE AND DATES COVERED</b> Annual Summary (1 Jul 98 - 30 Jun 02)
---	------------------------------------	--

<b>4. TITLE AND SUBTITLE</b> Human Progesterone A-Form as a Target for New Drug Discovery in Human Breast Cancer	<b>5. FUNDING NUMBERS</b> DAMD17-98-1-8070
--	---

<b>6. AUTHOR(S)</b> James W. Voltz D. McDonnell, Ph.D.	
--	--

<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Duke University Medical Center Durham, North Carolina 27710  E-Mail: <a href="mailto:donald.mcdonnell@duke.edu">donald.mcdonnell@duke.edu</a>	<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>
---	---

<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>
--	---

**11. SUPPLEMENTARY NOTES**

<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited	<b>12b. DISTRIBUTION CODE</b>
--	-------------------------------

**13. ABSTRACT (Maximum 200 Words)**

The role of the estrogen receptor (ER) in breast cancer has been suggested both by its ability to stimulate cell proliferation as well as the observation that ER is expressed in 60% of primary breast tumor biopsies but only in 6% of normal breast tissue. Drugs which interfere with ER activity such as the antiestrogen Tamoxifen have been only partially successful in the treatment of breast cancers emphasizing the need for new targets as well as new pharmacological agents against these targets. The observation that antiprogestins such as RU486 could function as antiestrogens suggested that the progesterone receptor (PR) could be a potential target in the treatment of breast cancers. The goal of this project was to elucidate the mechanism of hPR-A transdominant repression by characterizing potential hPR-A-interacting partners which are necessary for ER transcriptional activation. We anticipate that new pharmacological agents against these targets could be used to treat breast cancers which currently escape endocrine intervention.

<b>14. SUBJECT TERMS</b> Estrogen, NHERF, Breast cancer, PDGFR	<b>15. NUMBER OF PAGES</b> 22
	<b>16. PRICE CODE</b>

<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited
--	---	--	--

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

\_\_\_ Where copyrighted material is quoted, permission has been obtained to use such material.

\_\_\_ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

\_\_\_ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

\_\_\_ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

\_\_\_ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

\_\_\_ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

\_\_\_ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

\_\_\_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

---

PI - Signature

Date

## TABLE OF CONTENTS

SF298	.	.	.	.	.	.	.	.	.	p.2
Foreword	.	.	.	.	.	.	.	.	.	p.3
Table of Contents	.	.	.	.	.	.	.	.	.	p.4
Introduction	.	.	.	.	.	.	.	.	.	p.5
Body	.	.	.	.	.	.	.	.	.	p.6-9
Figures.	.	.	.	.	.	.	.	.	.	p.10-12
References	.	.	.	.	.	.	.	.	.	p.13-14
Appendix	.	.	.	.	.	.	.	.	.	p.15-22

## INTRODUCTION

The role of the estrogen receptor (ER) in breast cancer has been suggested both by its ability to stimulate cell proliferation as well as the observation that ER is expressed in 60% of primary breast tumor biopsies but only in 6% of normal breast tissue (1). Drugs which interfere with ER activity such as the antiestrogen Tamoxifen have been only partially successful in the treatment of breast cancers emphasizing the need for new targets as well as new pharmacological agents against these targets (2, 3, 4). The observation that antiprogestins such as RU486 could function as antiestrogens suggested that the progesterone receptor (PR) could be a potential target in the treatment of breast cancers (5, 6). To this effect our previous data show that the smaller isoform of human PR (hPR-A) functions as a ligand-dependent transdominant repressor of estrogen receptor (ER) transcriptional activity (7, 8). Although, the precise mechanism of hPR-A transrepression is not fully understood, we have recently identified an inhibitory domain (ID) located within the amino terminus of hPR-A, which permits hPR-A to transrepress ER transcriptional activity (9). Interestingly, although ID is contained within both PR isoforms, its activity is manifested only in the context of hPR-A, suggesting that hPR-A interacts with a set of cofactors that are distinct from those recognized by the larger isoform, hPR-B. To investigate potential role(s) of differential cofactor interactions, we looked at the ability of hPR-A and hPR-B to associate with different coactivators and corepressors and assessed the effect of these associations on the receptors' transcriptional activity (10). We also investigated whether any of these factors could be implicated in hPR-A-mediated transrepression of hER transcriptional activity (10). The goal of this project was to elucidate the mechanism of hPR-A transdominant repression by characterizing potential hPR-A-interacting partners which are necessary for ER transcriptional activation. We anticipate that new pharmacological agents against these targets could be used to treat breast cancers which currently escape endocrine intervention.

## BODY

### **I. Identification of an inhibitory domain within hPR-A required for transdominant repression of ER transcriptional activity.**

Human PR exists as two functionally distinct isoforms hPR-A and hPR-B (11). hPR-A is a truncated form of hPR-B lacking amino acids 1-164. In most cell- and promoter-contexts, hPR-B functions as a transcriptional activator, while hPR-A is transcriptionally inactive and functions as a ligand-dependent transdominant repressor of ER transcriptional activity (7, 8, 9). Unlike hPR-A, the A isoform of the chicken progesterone receptor (cPR-A), which shares 70% sequence homology with hPR-A, lacks this transdominant repressor function and acts as a strong activator of transcription (9). We have observed that the most extensive differences between the primary structures of the chicken and human PR-As are found in the amino terminal domains. Deletion of the first 140 amino acids from hPR-A ( $\Delta$ hPR-A) (Figure 1a) converted hPR-A into a transcriptional activator (Figure 1b) and abolished its ability to transrepress ER transcriptional activity (Figure 1c) suggesting that this domain is necessary for hPR-A transdominant repression (9). In addition, we found that this domain does not have autonomous activity when fused to a heterologous DBD suggesting that other sequences present within PR may be required for transrepression (9) (Figure 2).

### **II. The amino termini of hPR-A and hPR-B interact differentially with the carboxyl terminus of PR (hLBD) implying different receptor conformations.**

The presence of an inhibitory domain within human PR, whose function is masked in hPR-B, but not in hPR-A, suggests that the two receptor isoforms display different conformations within the cell which may allow for different cofactor interactions. This hypothesis is supported by our recent studies which analyzed the ability of separately expressed N- (PR-A and PR-B) and C-domains [hinge region plus ligand binding domain (hLBD)] of PR to interact in cells, by a mammalian two hybrid assay, and *in vitro* using purified expressed domains of PR (12). Specifically we found that the amino terminus of hPR-B, but not that of hPR-A, interacts efficiently with its hLBD both *in vivo* (Figure 3) and *in vitro* (Figure 4) in an agonist-dependent manner and

does not interact in the presence of antagonist RU486 (12). Together, these results suggest that the interaction between N- and C-terminal domains of PR is direct and requires an agonist induced conformational change in the LBD that is not allowed by antagonists. In addition, the more efficient interaction of the N-terminus of hPR-B, but not that of hPR-A, with the hLBD suggests that distinct structural differences between N- and C-terminal regions of hPR-A and hPR-B contribute to functional differences between hPR-A and hPR-B.

### **III. The two progesterone receptors exhibit different cofactor interactions which may explain the differences in their transcriptional activities.**

To determine whether the structural differences between the two receptors allow the receptors to interact with different cofactors, we looked at the ability of hPR-A and hPR-B to interact with various coactivators and corepressors (10). We demonstrated using a combination of *in vitro* and *in vivo* methodologies that the two receptors exhibit different cofactor interactions. Specifically, we showed using the mammalian two hybrid assay that the carboxyl terminus of the corepressor SMRT (C'SMRT), but not that of the corepressor NCoR ( $\Delta$ N4), interacts more strongly with hPR-A, than with hPR-B, and that this interaction is facilitated by ID (Figure 5). The physiological significance of this interaction was demonstrated using the dominant negative variant of SMRT, C'SMRT, to partially reverse hPR-A transdominant repression of ER transcriptional activity, directly implicating SMRT in the transrepression of ER activity by hPR-A. This was done by cotransfecting HeLa cells with ER, PR-A, and increasing concentrations of C'SMRT,  $\Delta$ N4, or full length SMRT in the presence of estradiol and RU486 (Figure 6). Increasing concentrations of full length SMRT did not reverse transrepression of ER activity by hPR-A (data not shown). In addition, we show that hPR-A, unlike hPR-B, is unable to efficiently recruit the transcriptional coactivators GRIP-1 and SRC-1 in the presence of agonist but not antagonists (10). This was determined by using the mammalian two hybrid assay and assessing the ability of the nuclear receptor interacting domains (NR) of SRC-1 and GRIP-1 fused to Gal4 DNA binding domain to interact with PR-A or PR-B fused to VP16 (Figure 7). We concluded from the above data that the inability of hPR-A, in contrast to hPR-B, to recruit coactivators, as well as its strong association with corepressor proteins, correlates with the differences in the transcriptional activities of the two PR isoforms.

#### **IV. Ongoing Studies**

Previously, we had proposed to use a modified version of the yeast two-hybrid screen to identify possible interacting partners of hPR-A, responsible for hPR-A transdominant repression of ER transcriptional activity (13). In order to do this, we integrated two PRE elements upstream of a LacZ gene into the yeast genome by homologous recombination and used full-length hPR-A as a bait, given the importance of receptor context for hPR-A mediated transrepression of ER activity (9). Unfortunately, when we tested for the intrinsic transcriptional activity of our bait construct we found that it to be high in most yeast strains tested, both in the presence of agonist R5020 and antagonist RU486 (data not shown). In addition to exhibiting high basal activity, our bait was also toxic to the yeast, when expressed at high levels. For these reasons, we decided to abandon the yeast two-hybrid screen and make use of phage display technology to look for potential peptides that distinguish between the two receptors.

Phage display technology has been used successfully in the past to search for peptide sequences that mimic endogenous protein-protein interactions (14, 15). We have used this technology successfully in the laboratory to screen for ER-interacting motifs using random peptide libraries (16). In order to identify peptides which bound specifically to hPR-A we screened six different random peptide libraries (L14, L15, L16, L17, L18, L19) against full-length hPR-A bound to R5020, purified from baculovirus. We immobilized 4nmoles of hPR-A onto 96-well plates. BSA was used as a negative control. Following this step, phage expressed peptides, from a random peptide library, were added to the wells and allowed to incubate for 1h at 25°C. The wells were then washed to remove any unbound phage. The bound phage from each library were eluted using a low pH buffer and saved for plaque purification. After plaque purifying the phage from each individual library we isolated the PR-A-specific phage using a phage ELISA assay to screen against both hPR-A and hPR-B in the presence of R5020. An anti-M13 antibody coupled to HRP was used to determine the specificity of the interaction. From this screen we have isolated phage which 1) bind specifically to hPR-A, and 2) bind to both hPR-A and hPR-B (Figure 8). We are currently sequencing the individual phage to obtain PR-A-interacting sequences which will be used to search the protein database.

#### **V. Conclusions**

The data presented within clearly explain why hPR-B acts a transcriptional activator of progesterone responsive promoters and why hPR-A is transcriptionally inactive. However, it remains to be determined how hPR-A and SMRT work to repress ER transcriptional activity. In conclusion, we believe that the structural differences between hPR-A and hPR-B may allow the A isoform of the receptor to interact with factors, which are not recognized by hPR-B, to form a complex which can interfere with ER-mediated transcription. Formal proof of this hypothesis awaits the identification of factors which can distinguish between the two isoforms of the human progesterone receptor.

FIGURES

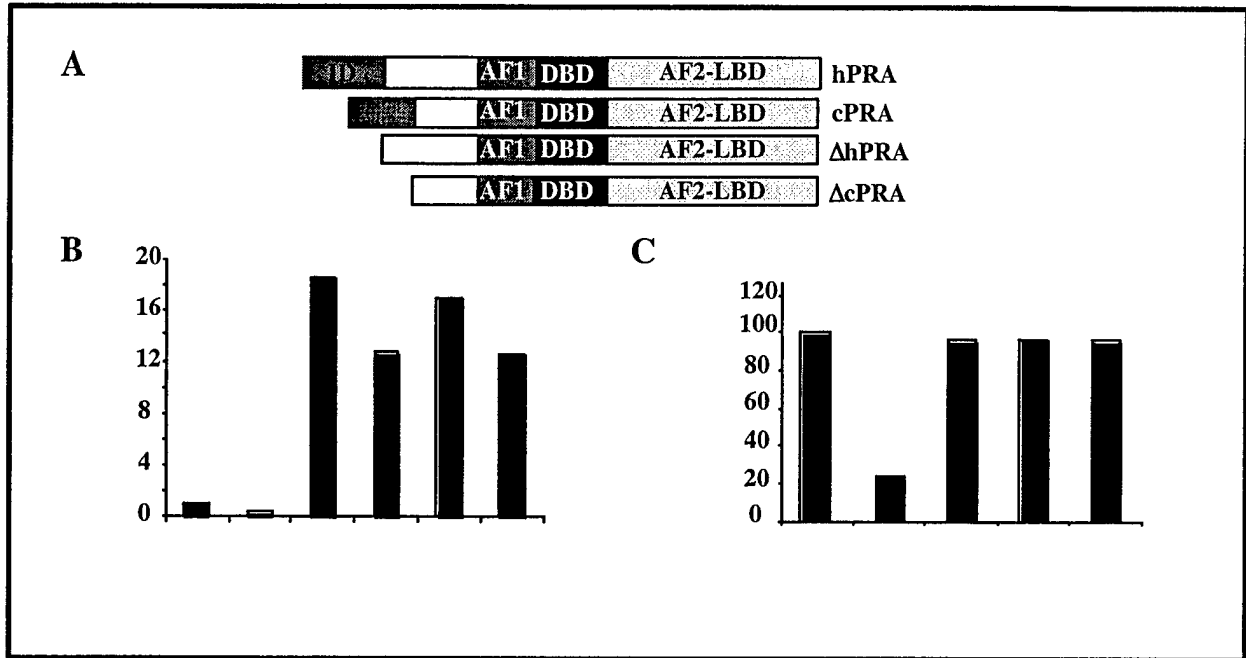


Figure 1

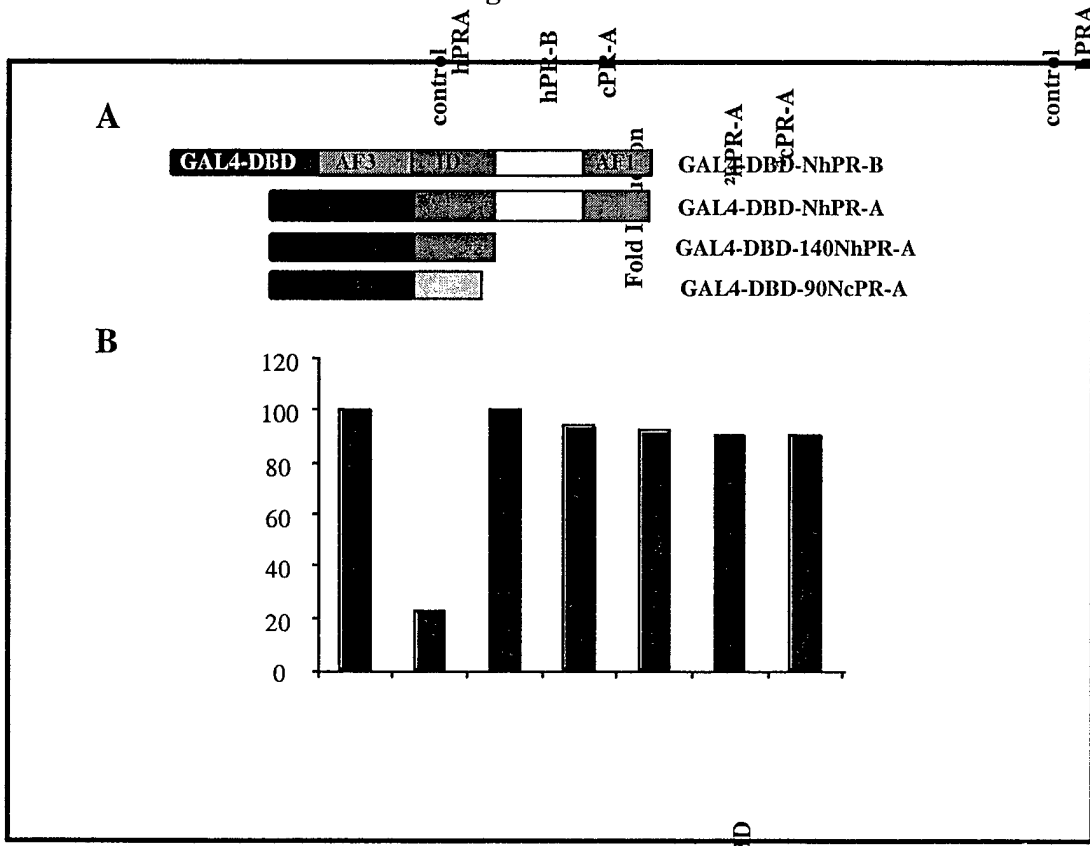


Figure 2

RA  
DBD

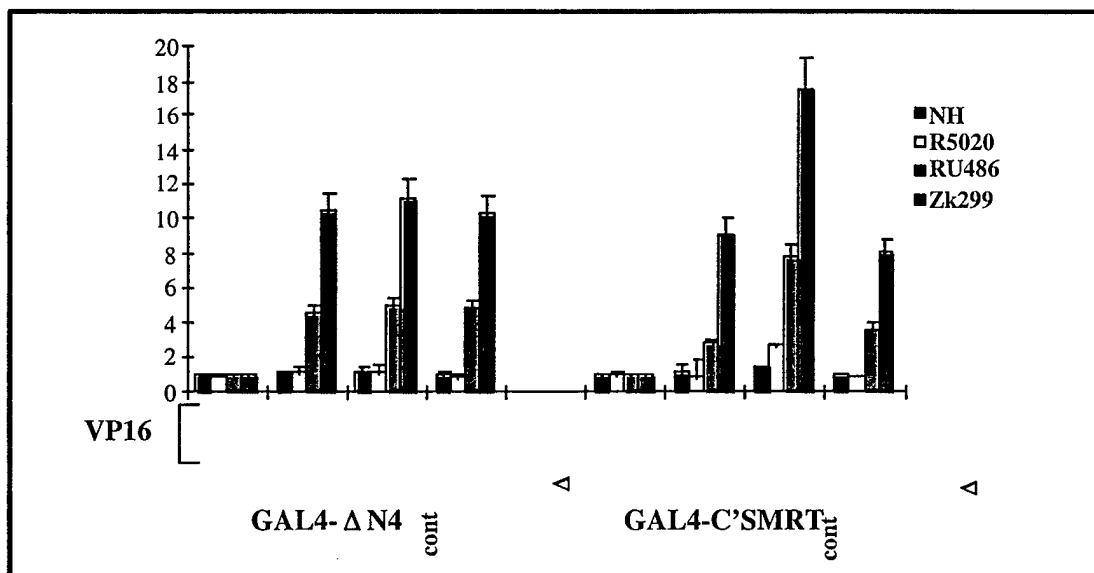


Figure 5

hPR-B hPR-A hPR-A hPR-B hPR-A hPR-A

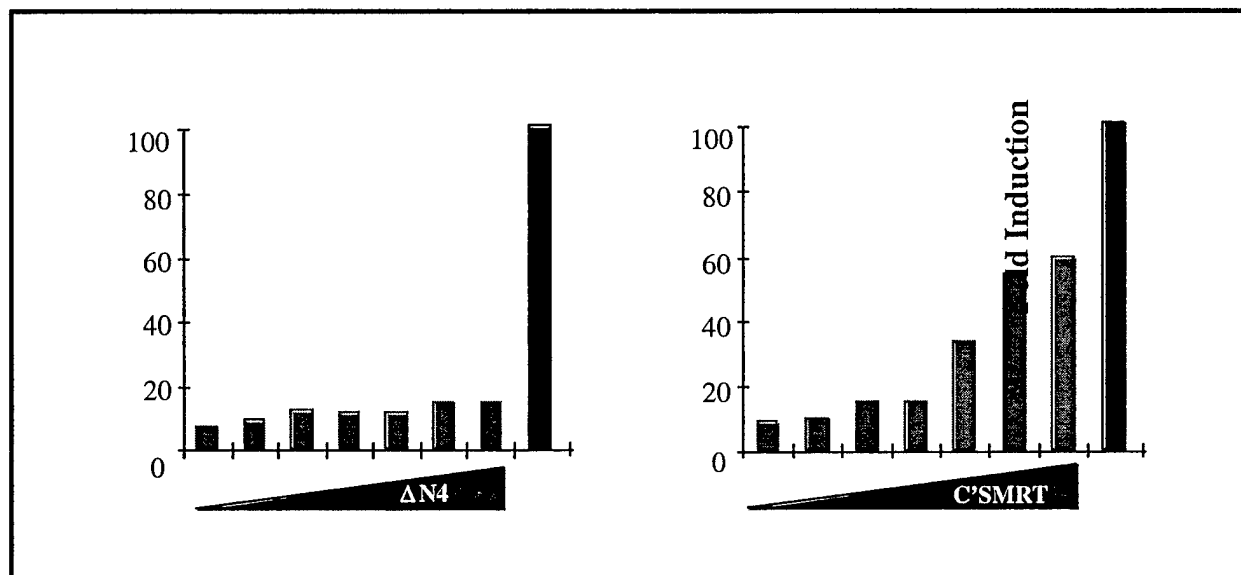


Figure 6

nt

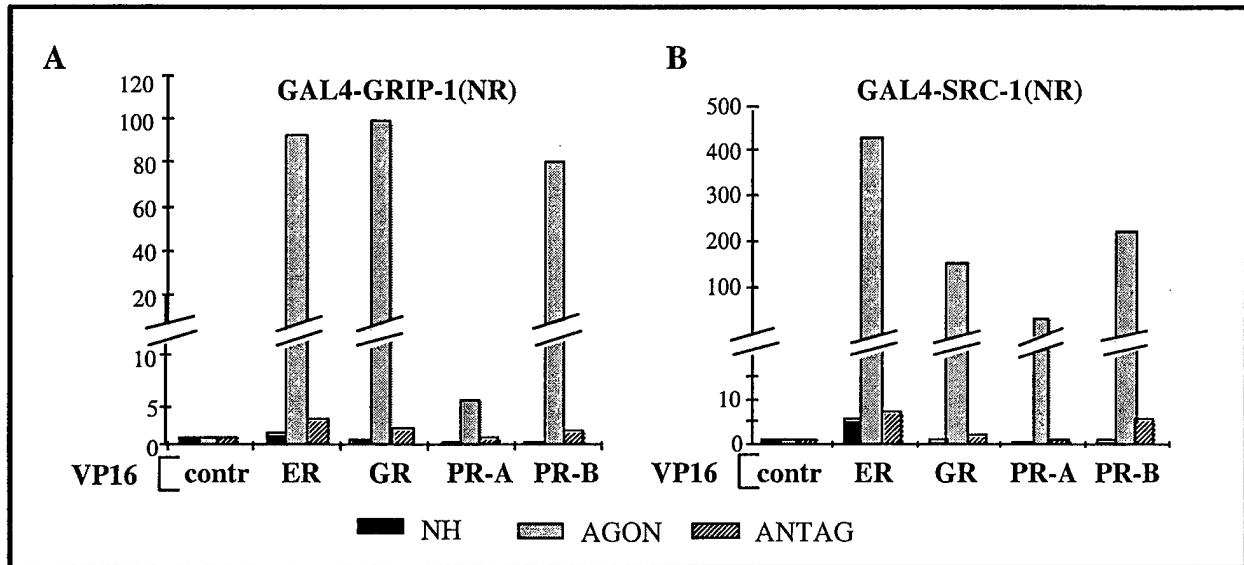


Figure 7

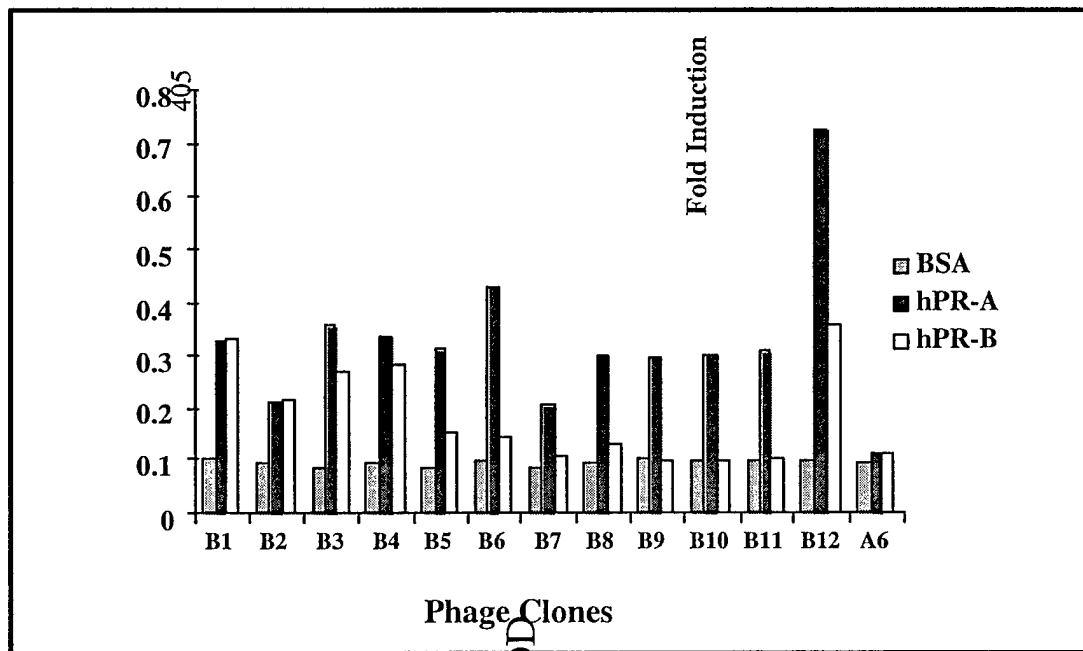


Figure 8

## REFERENCES

1. Scott, J. A., and McGuire, W. L. (1991) New Molecular Markers of prognosis in breast cancer. *Endocrine-Dependent Tumors*, ed. eds. Vogit, K.-D. and Knabbe, C. New York Raven Press. 179-196.
2. Love, R. R., and Koroltchouk, V. (1993) Tamoxifen therapy in breast cancer control worldwide. *Bull. World Health Org.* **71**: 795-803.
3. Jordan, V. C., Fritz, N. F., and Tormey, D. C. (1987) Endocrine effects of adjuvant chemotherapy and long term tamoxifen administration on node-positive patients with breast cancer. *Cancer Research* **47**: 624-630.
4. Suderland, M. C., and Osborne, C. K. (1991) Tamoxifen in pre-menopausal patients with metastatic breast cancer: a review. *Journal of Clinical Oncology* **9**: 1283-1297.
5. Romieu, G., Maudelonde, T., Ulmann, A., Pujol, H., Grenier, J., Cavalie, G., Khalaf, S., and Rochefort, H. (1989) The antiprogestin RU486 in advanced breast cancer: Preliminary clinical trial. *Bull. Cancer* **74**: 455-461.
6. Wolf, J. P., Hsiu, J. G., Anderson, T. L., Ulmann, A., Baulieu, E. E., and Hodgen, G. D. (1989) Noncompetitive antiestrogenic effect of RU486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrate monkeys. *Fertility and Sterility* **52**: 1055-1060.
7. Vegeto, E., Allan, G. F., Schrader, W. T., Tsai, M.-J., and O'Malley, B. W. (1992) Mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. *Cell* **69**: 703-713.
8. Vegeto, E., Shahbaz, M. M., Wen, D. X., Goldman, M. E., O'Malley, B. W., and McDonnell, D. P. (1993) Human progesterone receptor A form is a cell- and promoter- specific repressor of human progesterone receptor B function. *Mol. Endocrinology* **7**: 1244-1255.
9. Giangrande, P.H., Pollio, G., and McDonnell, D.P. (1997). Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J. Biol. Chem.*, **272**: 32889-32900.
10. Giangrande, P.H., and McDonnell, D.P. (1999). The Opposing Activities of the Two Isoforms of the Human Progesterone Receptor Are Due to Differential Cofactor Binding. (in preparation).
11. Lessey, B. A., Alexander, P.S., and Horwitz, K.B. 1983. The subunit characterization of human breast cancer progesterone receptors: Characterization by chromatography and photoaffinity labeling. *Endocrinol.* **112**: 1267-1274.

12. Tetel, M.J., Giangrande, P.H., Leonhardt, S.A., McDonnell, D.P., and Edwards, D.P. (1999). Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor *in vitro* and *in vivo*. *Mol. Endocrinol.* **13**:910-924.
  
13. Giangrande, P.H. 1998. Human Progesterone A-isoform as a Target for New Drug Discovery in Human Breast Cancer. Grant Number: DAMD17-98-1-8070. U.S. Army Medical Research and Materiel Command.
  
14. Pirozzi, G., McConnel, J.S., Uveges, A.J., Carter, J.M., Sparks, A.B., Kay, B.K., and Fowlkes, D.M. 1997. Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J. Biol. Chem.* **272**:14611-14616.
  
15. Sparks, A.B., Hoffman, M.G., McConnell, S.J., Fowlkes, D.M., and kay, B.K. 1996. Cloning of ligand targets: Systematic isolation of SH3 domain-containing proteins. *Nature Biotechnology* **14**:741-744.
  
16. Paige, L.A., Christensen, D.J., Grøn, H., Norris, J.D., Gottlin, E.B., Padilla, K.M., Chang, C., Ballas, L.M., Hamilton, P.T., McDonnell, D.P., and Fowlkes, D.M. 1999. Estrogen receptor modulators each induce distinct conformational changes in ER $\alpha$  and ER $\beta$ . *Proc. Natl. Sci. USA* **96**:3999-4004.

## APPENDIX

### 1) Research Accomplishments

- Defined the minimal domain of hPR-A required for transdominant repression of ER transcriptional activity
- Showed that the amino termini of hPR-A and hPR-B interact differentially with the carboxyl terminus of PR (hLBD) implying different receptor conformations.
- Showed that the two progesterone receptors exhibit different cofactor interactions.

### 2) Reportable Outcomes

#### Manuscripts

**Giangrande, P.H.**, Kimbrel, E.A., Edwards D.P., and McDonnell, D.P. (1999). The Opposing Activities of the Two Isoforms of the Human Progesterone Receptor Are Due to Differential Cofactor Binding. *Mol Cell Biol.* 2000 May;20(9):3102-15.).

\***Tetel, M.J., Giangrande, P.H.**, Leonhardt, S.A., McDonnell, D.P., and Edwards, D.P. (1999). Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor *in vitro* and *in vivo*. *Mol. Endocrinol.* 13:910-924. \*(Co-first authors)

Wagner, B.L., Pollio, G., **Giangrande, P.H.**, Webster, J.C., Breslin, M., Mais, D.E., Cook, C.E., Vedeckis, W.V., Ciblowski, J.A., McDonnell, D.P. (1999). The Novel Progesterone Receptor Antagonists RTI3021-012 and RTI3021-022 Exhibit Complex Glucocorticoid Receptor Antagonist Activities: Implications for the Development of Dissociated Antiprogestins. *Endocrinol.*, 140: 1449-1458.

**Giangrande, P.H.**, and McDonnell, D.P. (1999). The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by the same gene. *Recent Progress in Hormone Research: Proceedings of the 1998 Conference*, 54: 291-314.

**Giangrande, P.H.**; Pollio, G.; and McDonnell, D.P. (1998). Functional and pharmacological analysis of the A and B isoforms of the human progesterone receptor. *Schering Workshop*, **24**: 179-201.

**Giangrande, P.H.**; Pollio, G.; and McDonnell, D.P. (1997). Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J. Biol. Chem.*, **272**: 32889-32900.

Conference Presentations and Posters

**Giangrande, P.H.** and McDonnell, D.P. The Opposing Activities of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Cofactor Binding. American Association for Cancer Research, Steroid Hormone Receptors Symposium, Palm Springs, CA, 1999. (Travel Award)

**Giangrande, P.H.**, and McDonnell, D.P. The Differential Activity of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Co-repressor Binding. Duke University Graduate Student Symposium 1998. (Talk)

**Giangrande, P.H.**, and McDonnell, D.P. The Differential Activity of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Co-repressor Binding. Keystone Symposia, Lake Tahoe, NV; 1998.

**Giangrande, P.H.**, and McDonnell, D.P. Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. Duke University Graduate Student Symposium 1997.

**3) Copy of Cited Manuscripts and Abstracts (Attached)**

**American Association for Cancer Research, Steroid Hormone Receptors Symposium, Palm Springs, CA, 1999.**

The Opposing Activities of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Cofactor Binding.

Paloma H. Giangrande and Donald P. McDonnell . Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA.

In humans, the biological response to progesterone is mediated by two specific, high affinity nuclear receptors which are differentially expressed in target tissues. Both forms of the progesterone receptor (PR), hPR-A (94kDa) and hPR-B (114kDa), are derived from the same gene by alternative initiation of transcription. The only difference between the two receptor isoforms is that the first 164 amino acids of hPR-B are absent from hPR-A. These receptors are functionally different and have distinct roles in progesterone signaling. Specifically, we and others have observed that hPR-B functions as a transcriptional activator in response to agonist stimulation in all cell and promoter contexts examined. This is in contrast to hPR-A which is a transcriptional repressor and functions as a ligand-dependent transdominant repressor of hPR-B transcriptional activity. Of particular importance was the finding that ligand activated hPR-A can also inhibit the transcriptional activity of the estrogen (ER), androgen, glucocorticoid and mineralocorticoid receptors. Thus, hPR-A serves as a point of cross talk between the progesterone-signaling pathway and those regulated by other steroid hormones. The existence of two forms of PR has been documented in most species though the relationship between these receptors remains to be determined in most cases. Analysis of the properties of the chicken progesterone receptors (cPR) however, revealed that both cPR-A and cPR-B were efficient ligand dependent regulators of transcription. This was particularly interesting in view of the high degree of amino acid homology shared between the A-form of the chicken and human PRs. We took advantage of this finding to create a series of chicken/human receptor chimeras, the analysis of which permitted the identification of a specific transcription inhibitory domain located within the first 140 amino acids of hPR-A. Importantly, when transferred to the chicken receptor this inhibitory domain converted cPR-A into a transcriptional repressor. Previously, we have shown that the nuclear co-repressors NCoR and SMRT are important regulators of hPR-B mediated signaling. In the absence of hormone, or in the presence of pure antagonists, it was determined that these co-repressor proteins were able to interact with PR-B. Upon agonist binding however, a conformational change in the receptor occurred which favored the recruitment of co-activator proteins, and the subsequent displacement of co-repressors. These findings, coupled with the identification of an inhibitory domain within hPR-A, suggested that the differences in the transcriptional activity of the two PR-isoforms reflected differences in their ability to interact with co-activators and co-repressors. In support of this hypothesis, we have now shown that both forms of hPR are capable of interacting with SMRT and NCoR. However, the interaction of hPR-A with one of these co-repressors, SMRT, is much stronger than that observed with hPR-B. The physiological significance of this interaction was demonstrated by showing that expression of a dominant negative SMRT variant, cSMRT, reversed hPR-A mediated repression of both hPR-B and hER mediated transcriptional activity. Additionally, using both *in vitro* and *in vivo* methodologies, it was determined that hPR-B, but not hPR-A, interacts efficiently with the co-activators SRC-1 and GRIP. Based on these findings we propose that the ability of hPR-A to function as a transdominant repressor is a product of its enhanced corepressor binding affinity and its reduced affinity for co-activator proteins. Whereas these data clearly explain why hPR-A

is not transcriptionally active, it remains to be determined how the hPR-A/SMRT complex can transrepress the transcriptional activity of hPR-B and other steroid hormone receptors.

## Duke University Graduate Student Symposium 1998.

The Differential Activity of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Co-Repressor Binding

Paloma H. Giangrande and Donald P. McDonnell. Department of Pharmacology and Cancer Biology, Molecular Cancer Biology Program, DUMC, Duke University, Durham, NC 27710, USA.

In humans, the biological response to progesterone is mediated by two forms of the progesterone receptor (hPR-A; 94kDa and hPR-B; 114kDa). These two isoforms are transcribed from distinct estrogen-inducible promoters within a single-copy PR gene; the only difference between them is that the first 164 amino acids of hPR-B are absent in hPR-A. In most cell lines, hPR-A functions as a transcriptional repressor of progesterone-responsive promoters, whereas hPR-B functions as a transcriptional activator of the same genes. Interestingly, in these cell contexts, hPR-A also acts as a trans-dominant repressor of the transcriptional activity of other steroid hormone receptors.

In contrast to hPR-A, which functions predominantly as a ligand-dependent transcriptional repressor, we showed that the A isoform of the chicken PR (cPR-A) lacks this trans-dominant repressor function and is a transcriptional activator in all contexts examined. By constructing chimeras between the chicken and human PR we mapped the inhibitory function of hPR-A to the amino terminus of the protein. Although this inhibitory domain is present in hPR-B its activity is only manifested in the context of hPR-A.

The identification of a discrete inhibitory region within hPR-A whose activity is masked in the context of hPR-B, suggests that these two receptor isoforms may interact with different proteins (transcription factors, co-activators, co-repressors) within the cell. In support of this hypothesis, we have shown that the two isoforms of human PR are capable of interacting with the nuclear co-repressor proteins, SMRT and NCoR. Significantly, however, the interaction of hPR-A with the co-repressor SMRT is much stronger than that observed with hPR-B. Interestingly, we show that overexpression of a dominant negative SMRT (C' SMRT), but not a dominant negative NCoR ( $\Delta N4$ ), can reverse hPR-A-mediated transrepression. This important observation suggests that the ability of hPR-A to repress hPR-B transcriptional activity could occur as a consequence of hPR-B/A heterodimerization where the presence of SMRT in the complex prevents transcriptional activation. The observation that hPR-A also inhibits human estrogen receptor transcriptional activity, a receptor with which hPR-A is not able to heterodimerize with, suggests that there must be additional complexity.

**Keystone Symposia, Lake Tahoe, NV; 1998.**

The Differential Activity of the Two Isoforms of the Human Progesterone Receptor Can Be Explained In Part by Differential Co-repressor Binding.

Paloma H. Giangrande and Donald P. McDonnell Department of Pharmacology and Cancer Biology, DUMC, Duke University, Durham, NC 27710, USA.

In humans, the biological response to progesterone is mediated by two distinct forms of the progesterone receptor (hPR-A; 94kD and hPR-B; 114kD). These two isoforms are transcribed from distinct estrogen-inducible promoters within a single-copy PR gene; the only difference between them is that the first 164 amino acids of hPR-B are absent in hPR-A. In most cell lines hPR-A functions as a transcriptional repressor, whereas hPR-B functions as a transcriptional activator of progesterone-responsive genes. Interestingly, in these cell contexts, hPR-A also acts as a trans-dominant repressor of the transcriptional activity of other steroid hormone receptors.

In contrast to hPR-A, which functions predominantly as a ligand dependent transcriptional repressor, we showed that the A isoform of the chicken PR (cPR-A) lacks this trans-dominant repressor function and is a transcriptional activator in all contexts examined. By constructing chimeras between the chicken and human PR we mapped the trans-dominant repressor function of hPR-A to the first 140 amino acids of the protein. Interestingly, this trans-repression function is comprised not only of the "repressor domain" of hPR-A but also requires the context of the receptor in order to function.

The identification of a discrete inhibitory region within hPR-A, which is transferable to another receptor, implies that this region interacts with a set of transcription factors or adaptors which are distinct from those recognized by hPR-B. In support of this hypothesis, we have shown that the two isoforms of human PR are capable of interacting with the nuclear co-repressor proteins, SMRT and NCoR. Significantly, however, the interaction of hPR-A with the co-repressor SMRT is much stronger than that observed with hPR-B. This suggests, therefore, that the amino acid sequences in the amino terminus of hPR-B are important regulators of co-repressor interaction and that differential co-repressor association may explain in part the differential transcriptional activity of hPR-A and hPR-B. The identification of additional cell-specific adaptors will be required in order to better define the mechanism by which hPR-A modulates steroid hormone receptor transcriptional activity.

## Duke University Graduate Student Symposium 1997.

Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor.

Paloma H. Giangrande and Donald P. McDonnell. Department of Pharmacology and Cancer Biology, Molecular Cancer Biology Program, DUMC, Duke University, Durham, NC 27710, USA.

In humans, the biological response to progesterone is mediated by two distinct forms of the progesterone receptor (hPR-A; 94Kd and hPR-B; 114Kd). These two isoforms are transcribed from distinct estrogen inducible promoters within a single-copy PR gene; the only difference between them is that the first 164 amino acids of hPR-B are absent in hPR-A. In most cell lines hPR-A functions as a transcriptional repressor, whereas hPR-B functions as a transcriptional activator of progesterone-responsive genes. Interestingly, in these cell contexts, hPR-A also acts as a trans-dominant repressor of the transcriptional activity of other steroid hormone receptors.

In contrast to hPR-A, we have determined that the A isoform of the chicken PR (cPR-A) lacks this trans-dominant repressor function and is a transcriptional activator in all contexts examined. By constructing chimeras between the N-terminal domains of cPR-A and hPR-A we mapped the trans-dominant repressor function of hPR-A to the first 140 amino acids of the protein. Notably, when this "repressor" domain is placed onto cPR-A the activity of the latter changes from a transcriptional activator to a repressor. Interestingly however, this "repressor domain" is necessary, but not sufficient, for trans-repression as it is inactive when it is tethered to a heterologous protein. This suggests that the trans-repression function is comprised not only of the "repressor domain" of hPR-A but also requires the context of the receptor in order to function. The identification of a discrete inhibitory region within hPR-A which is transferable to another receptor implies that this region interacts with a set of transcription co-factors which are distinct from those recognized by hPR-B. The identification of these proteins is a crucial step in the definition of the mechanism by which hPR-A modulates steroid hormone receptor transcriptional activity.