

AD _____

Award Number: DAMD17-01-1-0431

TITLE: Regulation of Multidrug Resistance Phenotype and P-Glycoprotein Activity in MCF-7 Cells by the Epithelial Na⁺ Channel

PRINCIPAL INVESTIGATOR: Mouhamed S. Awayda, Ph.D.

CONTRACTING ORGANIZATION: Tulane University Health Sciences Center
New Orleans, Louisiana 70112-2699

REPORT DATE: August 2004

TYPE OF REPORT: Final

20050328 021

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.

REPORT DOCUMENTATION PAGEForm 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

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 2004	3. REPORT TYPE AND DATES COVERED Final (1 Aug 2001 - 31 Jul 2004)	
4. TITLE AND SUBTITLE Regulation of Multidrug Resistance Phenotype and P-Glycoprotein Activity in MCF-7 Cells by the Epithelial Na ⁺ Channel			5. FUNDING NUMBERS DAMD17-01-1-0431	
6. AUTHOR(S) Mouhamed S. Awayda, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tulane University Health Sciences Center New Orleans, Louisiana 70112-2699 <i>E-Mail:</i> mawayda@tulane.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Drug resistance is a process that occurs in a variety of carcinomas and especially in epithelial breast carcinomas. These carcinomas represent ~80% of all breast cancer types and are the subject of intense study. The origins of drug resistance in these cells are poorly determined. This proposal deals with examining the roles of the cell membrane and the properties of ion channels within this membrane in drug resistance. It is well known that the plasma membrane, through its role as a permeability barrier that defines and differentiates the intracellular from the extracellular one, plays a vital role in cell viability and survival to various noxious agents. However, the transport properties of breast epithelial cells and certainly those of cancerous origins are essentially undetermined. We propose to define these properties and to test the effects of transport alterations on cell viability and resistance to anthracycline antibiotics, agents which are widely used to combat breast cancer. Moreover, the routes of drug entry and exist across polarized cells (with apical and basolateral membranes) will be determined.				
14. SUBJECT TERMS Drug resistance, epithelial cells, ion channels, anthracyclines			15. NUMBER OF PAGES 6	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	3
Summary of Research efforts.....	3
Reportable Outcomes.....	5
Conclusions.....	5
Supported Personnel.....	5

Introduction:

This Idea Award project was initiated to test the hypothesis that the membrane ionic permeability affects cell growth in general, and the response of a model breast carcinoma cell line to drug treatment in particular. This represents the final report for the funding period of this grant and lists key findings and accomplishments as well as future directions.

The rationale behind this work was very straightforward. Epithelial breast carcinoma is one of the most common forms of breast cancer. It is a highly devastating disease with clear emotional as well as economic implications. One of the premier treatments for this disease is chemotherapeutics- namely the use of anthracycline antibiotics. A major shortcoming and the prime cause of failure of this treatment is the ensuing phenomenon of drug resistance. This process is attributed to the presence of specific proteins that transport the chemotherapeutic agents out of the cells. One such transporter is P-glycoprotein or P-gp1. This transporter shares homology with a family of proteins that include CFTR (Cystic Fibrosis Conductance Regulator). Indeed, CFTR is known to code for a membrane Cl⁻ channel. Moreover, CFTR exhibits negative regulation with ENaC (Epithelial Na⁺ Channel). This led us to the hypothesis that ENaC might participate in drug resistance either through effects on membrane voltage or through interaction with P-gp1 in its potential capacity to substitute for CFTR.

An additional factor for consideration was to identify the mechanisms of drug uptake and efflux in a model of breast epithelial carcinoma. Previous studies examining such issues have utilized non-polarized epithelial cells. Our vast experience with epithelia and epithelial transport have taught us that one cannot fully understand the physiology or pathology of such cells without examining polarized cells. This necessitates examining cells on permeable supports that allows the development of distinct apical and basolateral membranes. To our knowledge no previous studies have attempted to define the routes of drug movement across distinct apical and basolateral membranes of epithelia. Thus, our project was initiated by assessing such properties. This was followed by testing the interaction of ENaC and P-gp1 and the role of ENaC in drug uptake and efflux in polarized cells.

Summary of Research Efforts:

Utilizing the MCF-7 cell as a model of an epithelial breast carcinoma, we accomplished the following objectives:

1. Culture cells under conditions that allow for polarization and successfully describe the transepithelial routes of ion transport.

- a. Establishment of procedures to grow cells under polarized conditions
- b. Documentation of transepithelial ionic transport and validation of a functional apical membrane Na⁺ channel that is amiloride inhibitable.
- c. Verification that this channel is the same as the cloned ENaC via functional methods (single channel patch clamp analyses) and biochemical methods (Western blotting).

Reportable Outcomes:

1. 2001, Tulane Cancer Center. Mechanisms of Drug Resistance in MCF-7 Cells.
2. 2001, Sydney Australia. Invited presentation at the International Union of Physiologist (IUPS), Satellite Symposium "Transport Across Exocrine Glands".
3. 2002, Department of Physiology/Tulane Hypertension Center of Excellence. Mechanisms of Drug Uptake and Efflux in Breast Epithelia.
4. 2003, Tulane Cancer Center. Effects of Ion Transport on Cell Survival and Drug resistance in a Model of Epithelial Breast Carcinoma
5. Awayda M.S. Sodium Transport Via an Apical ENaC in Cultured Breast Epithelial Cells. In Revision (submission of a revised version is on hold awaiting the conclusion of experiments outlined above in *i* and *ii*.)
6. Awayda M.S., D. Wood, and I. Vukojic. Mechanisms of drug uptake and efflux across an epithelial breast carcinoma model. In preparation. (completion of this is also pending the completion of the experiments outlined above in *i* and *ii*.)

Conclusions:

Our initial focus was that drug efflux is an important mechanism which impinges directly on cell survival in response to chemotherapeutic agent treatment. In light of our new and exciting findings, we have now shifted some of our emphasis to understanding drug uptake. This important contribution has the potential to revolutionize how we think about drug resistance. These findings provide impetus for future studies documenting these uptake mechanisms. Thus, a new model of drug resistance may be warranted indicating that uptake of anthracyclines is not a strictly passive process, and that this may represent the target of new chemotherapeutic agents to treat such carcinomas aimed at further enhancement of uptake.

Supported Personnel:

Awayda M.S.	PI
Vukojic I	Research Associate
Woods D.	Student Researcher

Appendices:

None

Table of Contents

Cover.....	
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	

TITLE: PET Imaging of Breast Cancer using F-18 Labeled Choline Analogs

Principal Investigator: Mary Scott Soo, M.D.

Department of Radiology
Box 3808
South Hospital
Duke University Medical Center
Durham, N.C. 27710
919-684-7829

Co-investigators: Timothy DeGrado Ph.D., Eric L. Rosen M.D., Tim Turkington, Ph.D., R. Edward Coleman M.D., Lesa Kurylo

INTRODUCTION: Mammography has proven effective for reducing mortality from breast cancer, however detection of some lesions is limited by dense breast tissue that obscures the tumor, and the specificity of mammography is low. Functional imaging with positron emission tomography (PET) may help improve detection and diagnosis of breast cancer, although, the diagnostic accuracy of primary breast tumors has been found to be hindered by low glycolytic rates, and nonspecific uptake by nonmalignant breast tissue. Imaging with fluorocholine (FCH) may help to overcome these limitations. An efficient and practical synthesis of FCH has been developed at our institution. The purpose of the study is to evaluate the potential utility of F-18 labeled choline (FCH) as a positron emission tomography (PET) radiotracer for detection and diagnosis of breast cancer in women with highly suspicious breast lesions. Our goals are to determine: 1) the correlation between FCH uptake in primary breast cancer tumors and the histologic tumor type from subsequent pathology; 2) how FCH uptake in breast cancer tumors compares to F-18 2-fluoro-2-deoxy-glucose (FDG) (which is the current standard cancer imaging agent for PET) uptake in breast cancer tumors; and 3) if FCH can improve local staging of the breast in patients with recently diagnosed breast cancer (invasive or *in situ*).

BODY: To date, no subjects have been recruited or enrolled into this study. In order to satisfy all the requirements of the Human Subjects Protection Scientist for Human Subjects Approval prior to beginning the study, it has been required that we apply for an IND from the FDA for the experimental drug fluorocholine. While some progress had been made in the IND approval process, it is now apparent that it is unlikely that IND approval will occur in the near future, due to various issues. Therefore at this time we feel obliged to terminate this protocol and return the allotted funding.

KEY RESEARCH ACCOMPLISHMENTS: Progress toward IND final approval has been halted. Phase 1 studies cannot be performed at this time based on patent-related issues.

REPORTABLE OUTCOMES: Not applicable as above.

CONCLUSIONS: Due to a current failure in progress toward IND approval, at this time we feel we should terminate the project and are in the process of returning the funding to the Army.

REFERENCES:

DeGrado TR, Baldwin SW, Wang S, Orr MD, Liao RP, Friedman HS, Reiman RE, Price DT, Coleman RE. Synthesis and evaluation of 18F-labeled choline analogs as oncologic PET tracers. *J Nucl Med*, In press.

DeGrado TR, Coleman RE, Wang S, Baldwin SW, Orr MD, Robertson CN, Polascik TJ, Reiman RE, Price DT. Synthesis and evaluation of 18F-labeled choline as an oncologic tracer for positron emission tomography: initial findings in prostate cancer. *Cancer Res*, 61:110-117, 2001.

DeGrado TR, Orr MD, Wang SW, Price DT, Coleman RE, Baldwin SW. Structure-activity relationships for uptake of 18F-labeled choline analogs by prostate cancer models. *Proceedings of the 4th International Symposium on Radiohalogens*, Whistler BC, Canada, September 9-13, 2000.

DeGrado TR, RE Coleman, Baldwin SW, Orr MD, Wang S, Liao RP, Price DT. F-18 Labeled Choline Analogues as PET Imaging Probes of Prostate Cancer, *J Urol*, In press.

DeGrado TR, Coleman RE, Wang S, Price DT, Orr MD, Baldwin SW. Structure-activity relationships of 18F-labeled choline analogs for oncologic PET imaging. *J Nucl Med* 2001; 42:149P.

DeGrado TR, Baldwin SW, Orr MD, Wang S, Liao RP, Price DT, Coleman RE. Preliminary metabolic studies with [18F]fluorocholine (FCH), a novel oncologic probe for PET. *J Nucl Med* 2001; 42:149P.

APPENDICES: Not applicable