Award Number: DAMD17-98-1-8043

TITLE: Genetic Susceptibility to Cancer Chemotherapy in Human Breast Cancer

PRINCIPAL INVESTIGATOR: William Hait, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Medicine and Dentistry of New Jersey Piscataway, New Jersey 08854-5635

REPORT DATE: July 2000

TYPE OF REPORT: Annual

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.
We have focused on the ability to predict responsiveness to tubulin targeting chemotherapeutic drugs by understanding the role of p53 repression on the expression of proteins that regulate microtubule dynamics. We previously demonstrated that repression of microtubule associated protein 4 (MAP-4) produced tubulin depolymerization, sensitivity to vinca alkaloids, and resistance to taxanes. We built on this observation to design a phase I clinical trial to determine if this could be accomplished in and confirmed the ability to produce this effect in several human specimens. At the same time we studied the repression of stathmin, a cytosolic phosphoprotein which binds to and stabilizes tubulin heterodimers. Stathmin appears to be more sensitive to repression by p53 than MAP-4 and may lead to additional approaches to treatment of patients with breast cancer during the next grant period.
Table of Contents

Cover...........................................................................................................1
SF 298...........................................................................................................2
Table of Contents.........................................................................................3
Introduction.....................................................................................................4
Body................................................................................................................4-5
Key Research Accomplishments.................................................................5
Reportable Outcomes......................................................................................6
Conclusions......................................................................................................7
References........................................................................................................7-8
Appendices.....................................................................................................9-12
INTRODUCTION

We have focused on the ability to predict responsiveness to tubulin targeting chemotherapeutic drugs by understanding the role of p53 repression on the expression of proteins that regulate microtubule dynamics. We previously demonstrated that repression of microtubule associated protein 4 (MAP-4) produced tubulin depolymerization, sensitivity to vinca alkaloids, and resistance to taxanes. We built on this observation to design a phase I clinical trial to determine if this could be accomplished in patients. In the first nine patients treated with doxorubicin to induce DNA damage, stabilize p53, and repress MAP4, we confirmed the ability to produce this effect in several human specimens. In addition, by escalating the doses of the vinca alkaloid, vinorelbine, we will define a Phase II dose for the sequential combination. At the same time we studied the repression of stathmin, a cytosolic phosphoprotein which binds to and stabilizes tubulin heterodimers. Stathmin appears to be more sensitive to repression by p53 than MAP-4 and may lead to additional approaches to treatment of patients with breast cancer during the next grant period.

BODY:

Task 1. To determine whether loss of p53 function leads to increased expression of proteins that regulate microtubule dynamics in human breast cancer.

Task 2. To determine whether overexpression of proteins that regulate microtubule dynamics is predictive of response to tubulin targeting drugs.

We focused our attention during this grant period on the possibility that MAP-4 could be repressed by p53 induction in human breast cancer cell lines and in patients with breast cancer. We found mixed results in that several cell lines did not repress MAP4 following p53 induction by a variety of agents. We took several approaches to explain the variability between cell lines in their ability to repress MAP-4 after stabilization of wt p53. The first was to investigate the role of bcl-2, an antiapoptotic protein shown by Murphy and colleagues to block gene repression by p53 (Murphy et al., 1996). In these studies we first determined whether bcl-2 was overexpressed in cell lines without MAP-4 repression and found that this was generally the case. We next attempted to block the effect of bcl-2 on MAP-4 repression by down regulation of bcl-2 with retinoic acid (Toma et al., 1997), or destruction with bcl-2 antisense. In both cases we were unable to induce MAP-4 repression despite interfering with the function of bcl-2 (Alli, E. et al, unpublished).

During this grant period we translated our findings with MAP-4 into a phase I clinical trial designed to answer the question “does DNA damage induce wild type p53, repress MAP-4, and sensitize cells to vinorelbine”. Using escalating doses of doxorubicin to induce DNA damage, we found that it was feasible to carry out this protocol in breast cancer patients and that in several cases the expected biological response was observed.
(Bash et al., unpublished). We are nearing completion of this Phase I trial, and plan to enter phase II during the next year.

During this last grant period we also began to investigate the role of statmin, a second protein regulated by p53, to determine its role in regulating the sensitivity to tubulin targeting drugs. We turned our attention to statmin because Murphy et al., had shown that this gene was more sensitive to p53 repression than MAP-4. In addition, since statmin binds to tubulin heterodimers and inhibits microtubule polymerization, the balance between statmin and MAP-4 could determine the sensitivity of cells to tubulin targeting drugs. Using a panel of human breast cancer cell lines, we found that induction of wild type p53 decreased the cellular content of statmin and that this response was more predictable than the repression of MAP-4 (appendix 1). In addition, we found that cells with mutant p53 had increased content of statmin. To investigate the role of statmin in more detail, we transfected human breast cancer cell lines with a human statmin expression construct under the control of a CMV promoter and analyzed both sense and antisense transfectants. These studies demonstrated that cell lines with increased statmin had decreased microtubule polymerization as measured by immunofluorescent staining of β-tubulin. During the next grant period we will use cell lines stably transfected with statmin to investigate the effect of alterations in statmin on the sensitivity to tubulin targeting drugs used in the treatment of breast cancer.

Finally, our laboratory discovered that p53 was involved in the multidrug resistance phenotype through a series of experiments demonstrating that wild type p53 transcriptionally repressed mdr1, the multidrug resistance protein gene (Sullivan et al., 2000). This observation will now be extended to our studies of human breast cancer to determine whether or not p53 may have even greater effects on drug sensitivity than we initially hypothesized.

KEY RESEARCH ACCOMPLISHMENTS:

- Characterized the expression of statmin in a panel of human breast cancer cell lines and MAP-4 in several human specimens.
- Demonstrated the ability to repress statmin in breast cancer cell lines treated with DNA damaging agents.
- Created statmin transfectants to investigate the role of this p53-regulated protein in the sensitivity to tubulin targeting drugs used to treat breast cancer.
- Discovered that MRP was regulated by p53.

REPORTABLE OUTCOMES:

Manuscripts

Patients with Prostate Cancer and Other Advanced Malignancies J Clin Oncol 17:2213-2218, 1999.


Abstracts.


Development of cell lines,
- Created two stathmin transfectants in BT20 cells (Alli et al., unpublished).

Funding applied for based on this work:
NIH R03-CA82607
DOD in prostate cancer (in preparation)
CONCLUSIONS:

In conclusion, we have confirmed the importance of the role of proteins that regulate microtubule dynamics in human breast cancer cell lines and specimens and their regulation by p53. We have capitalized on these observations to develop a new clinical trial for patients with breast cancer. We demonstrated the sensitivity of stathmin to p53 repression and for the first time demonstrated the role of p53 in multidrug resistance.

REFERENCES:


Figure 1. Western blot analysis of p53, mdm2, p21, stathmin and β-actin levels of MCF7 (A) and ZR75-1 (B), two wild-type p53-containing human breast cell lines. MCF-7 cells were left untreated or treated with 1 μM Doxorubicin or 5 μM Bleomycin. ZR75-1 cells were left untreated or treated with 20 seconds of UV-C or 800 nM Bleomycin. 30 μg of protein was loaded to detect p53 and mdm2; 100 μg of protein was loaded to detect p21, stathmin, and β-actin.
Figure 2. Western blot analysis of stathmin and β-actin levels in mutant p53-containing human breast cell lines. 100 μg of protein was loaded.

Figure 3. Immunofluorescent staining of stathmin and polymerized microtubules in BT20 (A,B) and BT549 (C,D) cells. Stathmin, red. Microtubules, green.
Figure 4. Immunofluorescent staining of stathmin and polymerized microtubules in BT20 vector alone- transfected cells (A, B), BT20 stathmin transfected cells(C,D), BT549 vector alone- transfected cells (E, F), and BT549 stathmin antisense transfected cells (G, H). Stathmin, red. Microtubules, green.
Clones were isolated and screened by western blot analysis. 50 % of protein was loaded.

Murphy's vector alone using Lipofectamine 2000 transfection reagent (Gibco). Containing the full length human stathmin sequence (obtained by Maureen Murphy) or vector alone using Lipofectamine 2000 transfection reagent (Gibco). Grown to 90 % confluence and transfected with 10 μg of pcDNA3.1 (Invitrogen) grown to 90% confluency and transfected with 10 μg of pcDNA3.1 (Invitrogen).

Figure 5. BT20 cells were stably transfected with stathmin. Cells were plated and

**p-actin**

**stathmin**

<table>
<thead>
<tr>
<th>Vector + insert</th>
<th>Vector + insert</th>
<th>Vector alone</th>
<th>Vector alone</th>
<th>Vector alone</th>
<th>BT20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone#3</td>
<td>Clone#1</td>
<td>Clone#3</td>
<td>Clone#1</td>
<td>Clone#1</td>
<td></td>
</tr>
</tbody>
</table>