Award Number: DAMD17-01-1-0425

TITLE: An Anti-Oncogenic Role for Decorin in Mammary Carcinoma

PRINCIPAL INVESTIGATOR: Renato V. Iozzo, M.D.

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, Pennsylvania 19107

REPORT DATE: October 2003

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.
A significant proportion of human breast cancers overexpress ErbB2, a member of the receptor tyrosine kinase gene family that also includes the epidermal growth factor receptor (EGFR). Overexpression of ErbB2 correlates with increased metastatic potential and poorer prognosis. Agents that antagonize the activity of ErbB family members have obvious clinical implications. We have previously discovered that decorin causes a functional inactivation of the oncogenic ErbB2 in mammary carcinoma cells overexpressing ErbB2. This leads to growth inhibition and cytodifferentiation of mammary tumor cells and a concurrent suppression of their tumorigenic potential in vivo. We have successfully demonstrated decorin’s cytostatic effect both in vitro and in vivo with a metastatic breast cancer cell model. Thus, decorin gene therapy helps in retarding the growth of human tumors in immunocompromised animals and could represent a new independent or adjunctive therapeutic modality against cancer. We have additionally created a breast cancer cell line that contains the decorin transgene under the control of an inducible promoter. We plan on using this cell line to study decorin’s temporal effects on both primary tumor development as well as on tumor spread and metastases. Our ultimate goal is to prove decorin’s efficacy as a tumor suppressor and possible means of therapy for breast cancer.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>1</td>
</tr>
<tr>
<td>SF 298</td>
<td>2</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>4</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>5</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>5</td>
</tr>
<tr>
<td>Conclusions</td>
<td>5</td>
</tr>
<tr>
<td>References</td>
<td>6</td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION

A significant proportion of human breast cancers overexpress ErbB2, a member of the receptor tyrosine kinase gene family that also includes the epidermal growth factor receptor (EGFR). Overexpression of ErbB2 correlates with increased metastatic potential and poorer prognosis. Agents that antagonize the activity of ErbB family members have obvious clinical implications. We have previously demonstrated that decorin is a novel ligand for the EGFR. This interaction triggers a signaling cascade that leads to activation of MAP kinase and ultimately to growth suppression. In the preliminary data that support the basis of this proposal, we discovered that decorin causes a functional inactivation of the oncogenic ErbB2 protein in mammary carcinoma cells overexpressing ErbB2. This leads to growth inhibition and cytodifferentiation of mammary tumor cells and a concurrent suppression of their tumorigenic potential in vivo. We hypothesize that expression of decorin by breast carcinoma cells in an in vivo tumor model will result in inhibition of tumor growth. We further hypothesize that exogenous administration of the human decorin transgene via a viral or liposomal transfer vector will lead to growth slowdown and/or growth inhibition of already established xenograft tumors of human breast carcinomas.

BODY

We have made significant progress in several areas, including finding a suitable breast cancer model, treatment of the model in vitro and in vivo with adenoviral decorin, and in the production of a cell line containing decorin under the control of an inducible promoter.

Breast cancer tumor model
We have tested several cell lines for tumorigenic potential in mice and have decided to use the MTLn3 cell line (a kind gift from J.E. Segall, Albert Einstein Medical Center). The MTLn3 cell line is a rat breast adenocarcinoma cell line that can spontaneously metastasize in vivo to the lung, much like human breast cancers, and expresses moderate amounts of EGFR and high amounts of ErbB2 (~500,000 receptors/cell).

Inducible decorin promoter
We have created MTLn3 cells expressing decorin under the control of a Tet responsive element with the RevTet On System (Clontech). MTLn3 cell clones express decorin only in the presence of doxycycline, a harmless antibiotic. We have confirmed high levels of inducible decorin expression in several clones via Western blotting (Figure 1). Growth inhibition tests in vitro are pending, as is Western blotting to confirm the amounts and phosphorylation states of EGFR and ErbB-2. In vivo tests will begin shortly if results are favorable.

Adenoviral decorin vector
The MTLn3 cell line has been successfully growth-inhibited in vitro using adenoviral decorin. Treatment of MTLn3 cells with adenovirus expressing decorin resulted in approximately 50% growth inhibition after 72 hours in culture with MOI’s of as little as 0.1 (1 viral particle for every 10 cells). Decorin’s secretion into the local environment is a major asset in these experiments, as all nearby
cells can be affected from decorin, not only those cells successfully transduced. We have additionally treated MTLn3 tumors in vivo in nu/nu and SCID mice with adenoviral decorin. Tumor growth inhibition of 70% was observed after only two treatments of adenoviral decorin (5x10^6 pfu per treatment) following tumor induction directly at the breast with 10^6 MTLn3 cells (Figure 2). We have previously shown this adenoviral vector to be successful in treating other types of tumors in vivo in preliminary testing (Reed et al., 2002).

**AAV decorin vector**

Generation of an AAV vector contaqing decorin is still a priority. AAV (adeno-associated virus) is a powerful viral delivery method for gene therapy (During 1997, Samulski et al., 1999). We are looking into the AAV Helper Free System (Stratagene). The system employs the AAV2 serotype virus, which can use either of two receptors for viral entry into cells, making it more versatile than other serotypes. The system additionally features a β-globin intron to improve message lifetime in the cytoplasm. If this system looks promising, then we will clone decorin into the packaging plasmid and begin in vitro testing.

**KEY RESEARCH ACCOMPLISHMENTS**

- Use of MTLn3 breast carcinoma tumor model to generate tumors in vivo that spontaneously metastasize to the lungs.
- Successful treatment of MTLn3 tumors in vivo with adenoviral decorin.
- Creation of MTLn3 cell clones with decorin under the control of an inducible promoter (Tet On System).

**REPORTABLE OUTCOMES**

At this time there are no reportable outcomes.

**CONCLUSIONS**

The ultimate goal of this research is to prove that human decorin is a viable candidate or adjunctive candidate for treatment of certain breast cancers, and that it is possible to deliver the decorin transgene by one or more well-established transfer methods to achieve a positive clinical response. While we have experienced considerable success with adenoviral vectors containing decorin (Reed et al., 2002, and unpublished results), our ultimate goal is to produce a long-lasting expression vector that also is less immunogenic. AAV viral vectors meet both criteria, but our attempts with it unfortunately suffer from low levels of expression. Our current strategy, an improved AAV-2 vector containing a β-globin intron and having the ability to package larger amounts of DNA, should prove
advantageous in the near future. Meanwhile, we will continue our work with the MTLn3 tumor model and adenoviral decorin vector, looking further into metastatic spread and prevention or reduction thereof. Additionally, our inducible cell line is being prepared for in vivo testing shortly, allowing us to assess the temporal effect of decorin expression on breast cancer development and spread. We have already been able to show that our concept—that decorin has promise as a future candidate in the battery of treatments against breast cancer—and we hope to be able to go on to solve the problems of a superior delivery system. We also are planning on exploring the temporal effects of decorin expression on tumor growth and tumor metastasis, a serious problem in breast cancer, with our inducible promoter. Decorin has so far proven to have powerful antitumor properties while completely lacking in toxicity, an important point to keep in mind. The next several months have great promise.

REFERENCES


APPENDIX

---