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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.
The synthesis of the final target probe compound has been accomplished on a scale that should be large enough for initial biological testing. While attempts to finalize an in vitro cell culture assay for enhancement of ultrasound images are still ongoing, we have been able to generate spheroids with our experimental cell lines, perfect our gel matrix for in vitro testing, and detect our cells in this system using a commercially available contrast agent, perfluoro-octyl bromine (PFOB) as a standard.
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INTRODUCTION

Ultrasound imaging is useful in the detection, differential diagnosis and monitoring of treatment for many types of cancers. There remains, however, a need to improve upon the accuracy of ultrasound when deployed within the setting of breast cancer. The latter could reduce the need for more intensive diagnostic studies such as computed tomography, magnetic resonance imaging and radionuclide scans, as well as the need for other invasive, interventional procedures such as needle or open biopsy. One way to improve ultrasound imaging is to administer a chemical contrast agent that can enhance the magnitude of the ultrasound signal when in the vicinity of the cancerous tissue. One means of directing chemical agents to cancerous tissue is to incorporate molecular features that are specifically recognized by cancer cells compared to healthy cells. We intend to explore a distinct molecular address system that is known to become over-expressed in some types of human breast cancer tissue, by coupling it to another small molecule that has the potential to enhance the magnitude of the ultrasound signal. We will then test and compare the ultrasound images produced by this hybridized probe molecule in human cancer cell cultures that do and do not over-express and display this particular type of molecular recognition.

BODY

Background

Ultrasound or ‘Uls’ imaging is useful in the detection, differential diagnosis and treatment of many types of cancers including that of breast cancer. There remains, however, a need to improve upon the accuracy of Ultrasound imaging. One way to improve Ultrasound imaging is to administer a chemical contrast agent (Ultrasound Contrast Agent, UlsCA), particularly when the latter can also be targeted toward the tissue of interest. Over-expression of integrin adhesion molecules on breast cancer cells destined to undergo metastasis provides an opportunity to target them by utilizing the RGD peptide motif as an address component for a given molecular cargo. While many of the Ultrasound Contrast Agents are gases, perfluorinated chains of eight or more carbons can also serve in this capacity. Although the latter appear to be well-suited as molecular cargos for targeted delivery, this type of combination remains to be explored for eventual use in the clinic.

Relevance

Enhancing the accuracy of breast cancer-related Ultrasound would reduce the need for more intensive diagnostic studies such as computed tomography (CT), magnetic resonance imaging (MRI) and radionuclide (Rn) scans, as well as the need for other invasive, interventional procedures such as needle or open biopsy.
Rationale

We propose that it should be possible to improve the use of Ultrasounds to image certain types of breast cancer by administering an Ultrasound Contrast Agent (UlsCA) consisting of a perfluorinated hydrocarbon conjugated to an Arg-Gly-Asp peptide motif.

Objectives

1. Synthesize Arg-Gly-Asp-N-CH2CH2(CF2)7CF3 (“Model UlsCA”).
2. Establish MDA-MB-435 as an in-house cell culture line.
3. Compare the Ultrasound images of MDA-MB-435 versus MCF12A with and without the “Model UlsCA.”
4. Relative enhancement of the Ultrasound for MDA-MB-435 when the “Model UlsCA” is present will constitute a ‘proof of principle’ at the in vitro level and thus become the basis for a major grant submission and broader investigation of this topic.

Methods

Approximately 250 mg of the “Model UlsCA” will be prepared by using standard, solution-phase, peptide coupling reactions starting with di-protected Arg. The final product will be characterized by MS, NMR (proton and fluorine), HPLC and elemental analysis. MDA-MB-435, an estrogen-independent breast cancer cell line that is known to express high levels of the integrins (5), will be purchased and added to our panel of in-house cell cultures such as MCF12A which will be used as a comparative control since it is a non-cancer but immortalized breast epithelial cell line. Ultrasound measurements will be done with a small unit initially designed to be used for rodents but also suitable for use with solution samples. A simultaneous or directly competing comparison will be accomplished via suspending the two cell types in solutions on opposite sides of a dialysis membrane. Side-by-side comparisons will be accomplished by using a plate/rinse off approach followed by Ultrasound measurement. Cells types can be compared to themselves with and without the “Model UlsCA,” as well as to each other with and without the “Model UlsCA.”

KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES

Although this grant was awarded on-schedule, there was a significant delay in our undertaking of the work due to the fact that we needed to staff both the postdoctoral and graduate student participants, both of which took longer than anticipated. Thus, the project did not get underway in a really meaningful manner until early 2007. Note that we also requested a “no-cost-extension” (NCE) near the end of the grant’s initial one-year anniversary. This report summarizes all progress to date.

In terms of the objectives listed above, we have now synthesized the final target probe compound on a scale that should be large enough to eventually allow for its initial biological assessment. Our successful synthesis scheme is attached as Appendix 1. We have established the MDA-MB-435 breast cancer cell line in our lab. While we still have to test our final target probe, we have recently made significant progress with our Ultrasound assay at the in vitro level. We have been able to detect a clearly discernable level of contrast with commercially available perfluorinated contrast agent (PFOB) when
imaged with the breast cell lines, MCF7, MCF12A, and NCI/Adr (Figures 1A and 1B), in our in vitro ultrasound system. Currently, we are testing MDA-MB-435 spheroids with PFOB (Figure 2) and will be moving into our final target probe within the next month. Since we are familiar with establishing in vivo tumor-implant models, we may try to assess our target probe using this model in order to move it into the next phase of clinical development. However, we will need to perform scale-up syntheses of target probe compound to generate enough probe for the in vivo experiments. Note that in addition to this summary report, we presented our results at the ‘Era of Hope’ conference in June, 2008 (6). A copy of our poster is attached as Appendix item 2.

Figure 1. Breast cancer cell spheroids with and without PFOB as a non-specific contrast agent for ultrasound analysis. (A) in vitro gelatin assays detecting PFOB in NCI/Adr and MCF7 breast cancer cell lines. The first panel depicts no cells with PFOB (left well) and no PFOB (right well); the second panel shows NCI/Adr cells without (left well) and with (right well) PFOB; third panel shows MCF7 without (left well) and with (right well) PFOB. (B) The quantitative results of the ultrasound images with background subtracted.

<table>
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<tr>
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<th>No PFOB n=2</th>
<th>With PFOB n=2</th>
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<th>X BG</th>
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<td>72.7</td>
<td>30.2</td>
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Figure 2. MDA-MB-435 breast cancer cells with (right well) and without (left well) contrast agent (PFOB).

Conclusion

Although off to a late start and having run into some significant hurdles in terms of the biological assay, we are optimistic that we will be able to accomplish the overall goal of our proposed research, namely to ascertain if tumor interfaces might be better defined with ultrasound by deploying tumor specific contrast enhancement agents. Toward that end, we plan to keep working on this project until we have at least accomplished the appropriate tests in such a fashion so as to pass judgment on the feasibility of such a diagnostic method even though the project’s grant has now been ‘officially’ ended.
REFERENCES

1. Personal communications with colleagues at a neighboring teaching and research hospital.


Appendix 1. Schematic of the synthesis of the targeted ultrasound contrast agent.

**SYNTHESIS OF RGD TRIPEPTIDE AS ADDRESS SYSTEM**

![Chemical structure](image)

(a) CDI, DCM, DIPEA; (b) 1:1 TFA/DCM; (c) i- DCC, BnOH, DMAP; ii- 10 eq. Octanethiol, DBU; Fmoc = 9H-Fluorenyl-9-methoxycarbonyl.

**SYNTHESIS OF ULTRASOUND IMAGING ENHANCER AS CARGO COMPONENT (CA)**

![Chemical structure](image)

(a) EtOAc, reflux, 70%; (b) i-10% Pd/C, H2, 30 psi, MeOH, 10 h, 95%; ii-MeOH/HCl (c) R5 (see above), CDI, DCM; (d) 10% Pd/C, H2, 25 psi, MeOH, 36 h.
Ultrasound Imaging for Breast Cancer

Jill A. Trendel, Mohammad El-Dakdouki, Nicole R. Ellis, Jeffrey G. Sarver and Paul W. Erhardt

INTRODUCTION

Although generally useful in cancer detection, diagnosis and therapy, there remains a need for improvements in accuracy of imaging methods. The current standards of care for breast cancer detection include x-ray mammography followed by biopsy of abnormal tissue. New imaging methods are being developed to improve accuracy in vivo imaging of breast cancer. One way to improve US imaging is to administer a chemical contrast agent that can enhance the magnitude of the ultrasound signal when in the vicinity of the cancerous tissue.

RESULTS

Using perfluoro-octyl bromide (PFOB), a previously studied contrast agent, we have validated our in vivo gel method using breast cancer cell lines. Image analysis shows that the contrast is enhanced by chemotherapy treatment. Gel analysis shows that the contrast is enhanced by chemotherapy treatment.

SYNTHESIS OF RGD TRIPEPTIDE AS ADDRESS SYSTEM

\[
\begin{align*}
\text{F} & \quad \text{COO} & \text{tBu} \\
\text{NH} & \quad \text{2} \\
\text{CA} & \quad \text{CDI, DCM; (d) 10% Pd/C, H}_{2} & 25 \\
\end{align*}
\]

Ultrasound imaging is useful in the detection, differential diagnosis and monitoring of breast cancer; however, its use is limited by the need for improved contrast agents. New imaging methods are being developed to improve accuracy in vivo imaging of breast cancer.

SYNTHESIS OF ULTRASOUND IMAGING ENHANCER AS CARGO COMPONENT (CA)

Using 10% palladium with 5% sodium under an atmosphere of hydrogen, we have synthesized an octanethiol (Fmoc) with a cargo that is anticipated to be able to enhance US imaging signals. Until the later is a need to improve the accuracy of ultrasound (US) when deployed within the setting of breast cancer.

FUTURE DIRECTIONS

Once we have demonstrated that our compounds have enhanced contrast, we will proceed to a clinical trial in vivo imaging of breast cancer.

REFERENCES


RESEARCH FUNDING BY

The United States Department of Defense W81XWH-06-1-0595

Appendix 2. Poster presentation at the Era for Hope conference, Baltimore, Maryland, June 2008.