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PRINCIPAL INVESTIGATOR: Mark A. Meighan, Ph.D.

CONTRACTING ORGANIZATION: University of Missouri-Columbia
Columbia, Missouri 65211

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6. AUTHOR(S):
Mark A. Meighan, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Missouri-Columbia
Columbia, Missouri 65211

E-Mail: meighanm@missouri.edu

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Breast cancer is the number one cause of death amongst cancer in women. New methods for early detection, diagnosis and treatment of cancer are always sought. In this report we show ongoing progress in the use of phage display libraries to isolate peptides and antibody fragments that target tumor material in an in vivo setting. Specifically we address the isolation of a number of phage peptide sequences and note that those isolated appear to be non specific and bind or 'stick' to non-tumor material. Further investigation is needed to determine if this is indeed the case.

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Phage Fab Display Selection In Vitro and In Vivo: Novel Means to Identify New Breast Cancer Avid Compounds

Year 3 Annual Report

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Introduction

Breast cancer kills more American women each year than any other cancer (1), and develops from normal breast epithelium through several stages. 'Invasive' breast carcinoma occurs when the epithelial cells invade the surrounding stroma (2), however the exact timing of the changing of the cells from benign to malignant remains unknown. Preinvasive lesions as well as some breast tumors may be detected by mammography, however many women with breast cancer remain undiagnosed using mammography alone. Alternative detection and diagnostic and therapeutic strategies are always sought. In this research we wanted to use the power of phage display technologies in an attempt to isolate antibody fragments and peptides that bind or target breast cancer tumor xenografts in an *in vivo* setting.

Body

The relevance of this research is that affinity selection of antibody fragments (Fab) or peptides that bind breast cancer cells *in vitro* and tumor material *in vivo* will allow us to develop small tumor-avid molecules capable of targeting breast tumor material from a wide variety of sources. These Fab/peptides will be assessed as to their usefulness as diagnostic and imaging tools. Our third year progress is summarized below.

Aim 1: To isolate Fab molecules that bind to breast cancer cell surface antigens using phage display technology and breast tumor cell lines *in vitro*

As previously reported, we had problems isolation stable Fab Phage in an *in vitro* setting and this approach was abandoned at this stage.

Aim 2: To isolate Fab molecules that bind breast tumors and their metastases using phage display technology utilizing an *in vivo* strategy

As previously reported breast tumor xenografts were established in mice using breast cancer cell line. Solid tumors were visible 4-8 weeks post injection. Several rounds of biopanning were achieved using an in vivo strategy as previously reported. To recap on the biopanning approach it appeared that the titer for phage that bound tumor material was increased at the expense of phage binding normal tissues and vasculature (Figure 1).

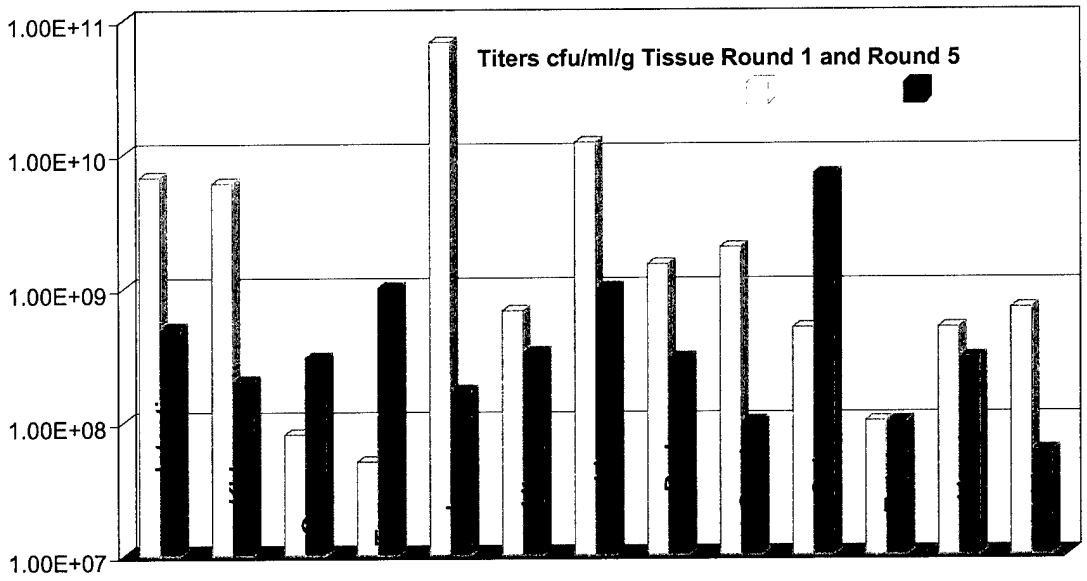

Figure 1
Fab-Phage Titers Round 1 and Round 5
(expressed as colony forming units (cfu) per ml per mg tissue)

It is interesting to note that the decrease in titers for the various tissues was not as dramatic as one would have liked. Much of this was probably due to physical trapping of the phage rather than specific binding.

Monoclonal populations of phage were produced and used for sequencing of the Fab insert. To our dismay DNA sequencing revealed that, for unknown reasons, the DNA encoding the Fab insert was unstable over the rounds of panning or that we isolated a predominant phage that had no insert. An attempt was made to sequence phage from round 2 of panning. While we did indeed obtain some sequence data – no predominant sequences or sequence similarities were noted.

A repeat set of biopanning gave similar results, problems and conclusions.

At this point was decided that we would use a different phage display library that displayed random 15-mer peptides on the surface of the phage. This library appears to be much more stable and its use is much more straightforward. Essentially, the *in vivo* biopanning technique using peptide libraries is the same. Approximately 10^{11}-10^{13} phage particles were injected into the tail vein of mice bearing tumor xenografts. After 1 hr, mice were sacrificed and perfused with 60 ml of phosphate buffered saline. Tissues were dissected and snap frozen in liquid nitrogen.

Tissues sample were weighed and washed in a similar fashion to that using the Fab phage display library. Phage were eluted and titered by infection into *E.coli* bacteria. *E. coli* infected with phage were amplified and phage precipitated from this for further rounds of panning. A similar phage titer profile was obtained to that seen in the Fab phage

biopanning (data not shown). After several rounds of panning, individual monoclonal phage poplulations were produced and samples were analyzed by DNA sequencing. It appeared (as predicted) that the peptide-phage library was much more stable than the Fab-phage library and sequences were attainable from all rounds of biopanning.

From the final round of biopanning, we sequenced a number of phage to determine if there were any predominant sequences. The table below shows several of the peptide sequences obtained.

```
>P10 ITKNNIPIIRSTSTR
>P11 PQTPNSPTPILKRLX
>P12 TRKMRRTSLKQPNIT
>P13 NPPKTSPLSNRQMRH
>P14 MPPRKRISHLNHSTS
>P15 RSSRPTSPTKKLLRP
>P16 RSXRPTSPTXKLLRP
>P17 SHRRSIKLPSRTLTL
```

The most predominant sequence was that represented by P15 – RSSRPTSPTKKLLRP and represented some 60% of the clones sequenced. The other sequences appeared more random in nature but some sequence similarities were detected. However, previous work carried out in our lab and others have shown that many peptides containing Arginine (R) and Lysine (K) residues have a tendency to be 'sticky' and are often isolated in biopanning experiments but have little or no specificity to the intended target(s). This indeed turned out to be the case. Utilizing a number of binding assays such as dot-blotting where we spot tumor material and non-tumor material onto nitrocellulose, we found similar binding intensity with the phage indicating that the phage are either just 'sticky' or recognize a similar target in both samples. Either way, unless the peptide/phage can be used to discriminate between tumor and non-tumor it is unlikely to be of use in the detection or therapy of cancer.

Work is on going to determine if any of the phage isolated bind to tumor material only and not to non-tumor material.

Key Research Accomplishments

Five rounds of biopanning with random 15-mer peptide phage display library.
Over one hundred clones sequenced.
One predominant peptide sequence obtained.
Phage/peptide did not distinguish between tumor and non-tumor material.

Reportable Outcomes

Poster Presentation at the 2002 Era of Hope Symposium, Orlando Florida

Conclusion

The project ran into difficulties early on with the finding that the Fab-phage display library being used was not very stable in an *in vivo* setting and that the displayed fragment or that the DNA encoding for it was somehow excised out of the phage. Or, alternatively that non-Fab displaying phage particles were propagated or isolated more readily than those phage displaying Fabs.
More encouraging results were obtained when we directed our approach to using random-peptide libraries. Here we were able to successfully carry out five rounds of biopanning *in vivo* and to obtain readable sequence data at the end. A predominant clone/sequence was isolated but initial indications are that the peptide/phage does not bind to or recognize a target specific to tumor material but also bind to non-tumor tissue (data not shown). Further work is needed to characterize this sequence and determine its binding target (if any). Radiolabelling of phage/Fabs/peptides has not been addressed up to this point.

Phage display technology is still a very powerful tool to isolate small molecules that bind any given target. The search is still on in this laboratory to isolate peptides and antibody fragments that bind tumor materials.

References

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