

PEPTIDE-MEDIATED MOLECULAR IMAGING OF INFLAMMATORY BREAST CANCER

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INTRODUCTION

Inflammatory breast cancer (IBC) is a subtype of breast cancer that has a frequent association with metastatic disease and a poorer prognosis than comparative non-inflammatory Stage III breast cancers. A review of the Surveillance, Epidemiology and End Results (SEER) database reveals the incidence of IBC is also increasing in a more significant pattern than non-inflammatory forms of breast cancer, prompting an urgent need to identify IBC-specific 'theranostics'.

Inherent receptor/ligand interactions that can occur on the surface of tumor cells can act as a dynamic molecular address that can enable targeted delivery of drugs and imaging agents to tumors. We hypothesize that such molecular addresses within IBC can be exploited for ligand-based imaging and early detection of disease sites. As a part of our ongoing studies we have identified, characterized and evaluated peptides that can target GRP78, a stress-response protein that becomes elevated in IBC and during metastatic progression (Figure 1). Indeed, we found that GRP78-targeting peptides can bind to and internalize within IBC cells. Further, using fluorescently-tagged phage variants, we prove that these peptides mediate homing towards IBC tumor xenografts *in vivo*. Taken together, we demonstrate a receptor/ligand system that can be used for imaging and therapeutic targeting of IBC tumors *in vivo*.

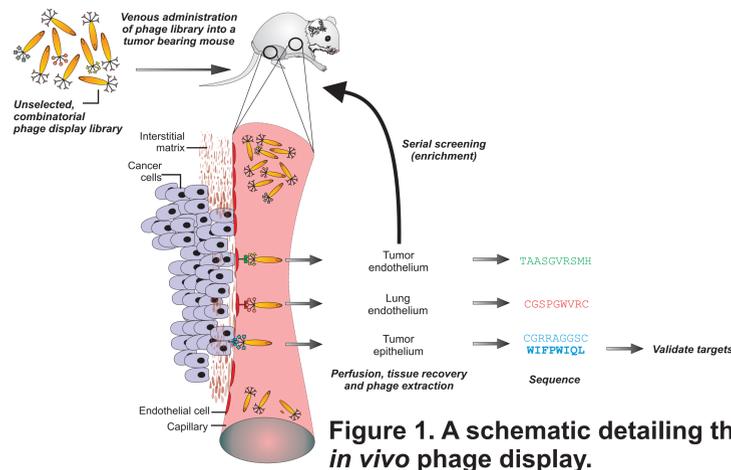


Figure 1. A schematic detailing the process of *in vivo* phage display.

Figure 2. WIFPWIQL-phage preferentially binds to inflammatory breast cancer cells *in vitro*. The ability of the peptide WIFPWIQL to bind to, and internalize in breast cancer cells was assessed by phage display. Briefly, filamentous phage displaying either WIFPWIQL or 'insertless' control were incubated on a panel of breast cancer cells. Subsequently, the fraction of cell bound/internalized phage was separated and quantified by bacterial infection and titration. Results displayed are the mean fold values (+/- SEM) compared to 'insertless' controls. IBC and IBC-like tumor lines, SUM149, SUM190 and EF43-fgf4; * P < 0.05, Student's t-test.

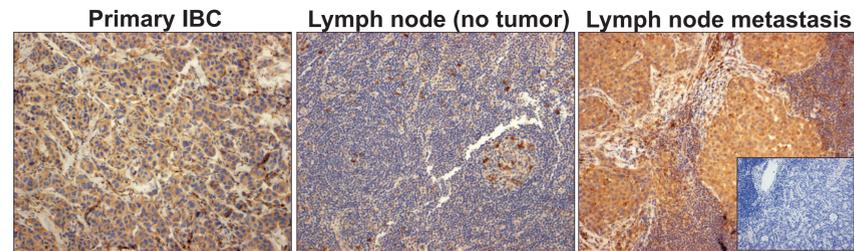
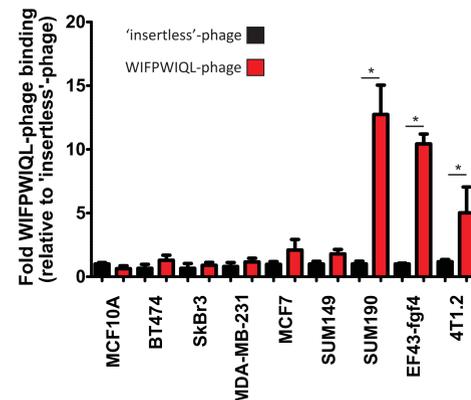


Figure 3. GRP78 is abundantly expressed in clinical IBC tumor samples. GRP78 expression was detected in epithelial cancer cells within both primary (left panel) and secondary (right panel) human IBC tumors. Tumor-free lymph nodes (middle panel) displayed minor GRP78 expression in germinal centers. Right panel inset, metastasis-positive lymph node stained with an isotype antibody control.

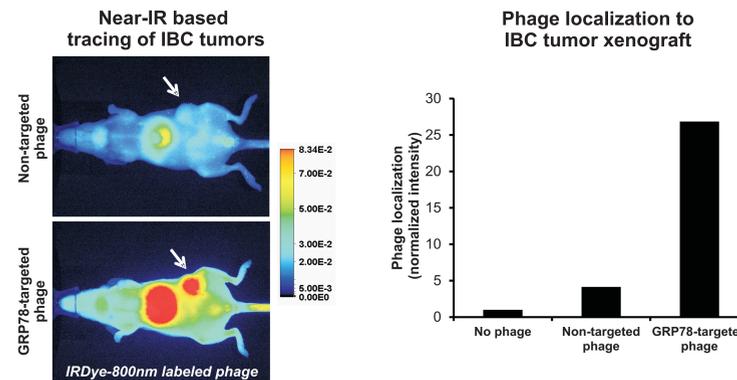


Figure 4. Molecular imaging of GRP78-targeted phage in mice bearing SUM190 inflammatory breast tumors. Mice were imaged 24 hours following an injection of two, dual-labeled phage species. IBC tumor-localized 800nm signal was only attained in mice receiving labeled WIFPWIQL-phage (arrow). Obtaining the relative fluorescent values can provide a measure of targeted phage accumulation in IBC tumors.

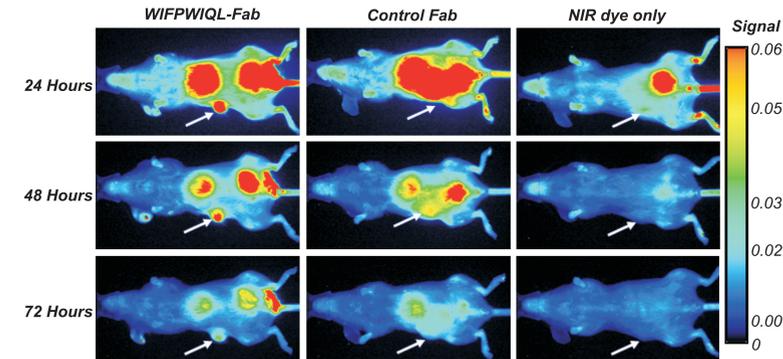


Figure 5. Utility of a peptide (WIFPWIQL)-engrafted antibody for the detection of EF43-fgf4 mammary tumors *in vivo*. Briefly, the GRP78-targeting peptide was grafted into the HCDR3 region of a human antibody-fragment (Fab). This antibody demonstrated specific binding to recombinant GRP78 by ELISA and could bind to the cell surface on GRP78-positive DU145 cells (data not shown). Equivalent amounts of NIR-dye labeled WIFPWIQL-Fab or nontargeting-Fab were administered *i.v.* to mice bearing EF43-fgf4 tumors, and the antibody traced over 72 hours.

Figure 6. BMT-78 kills IBC cells *in vitro* and inhibits tumor growth in a preclinical model.

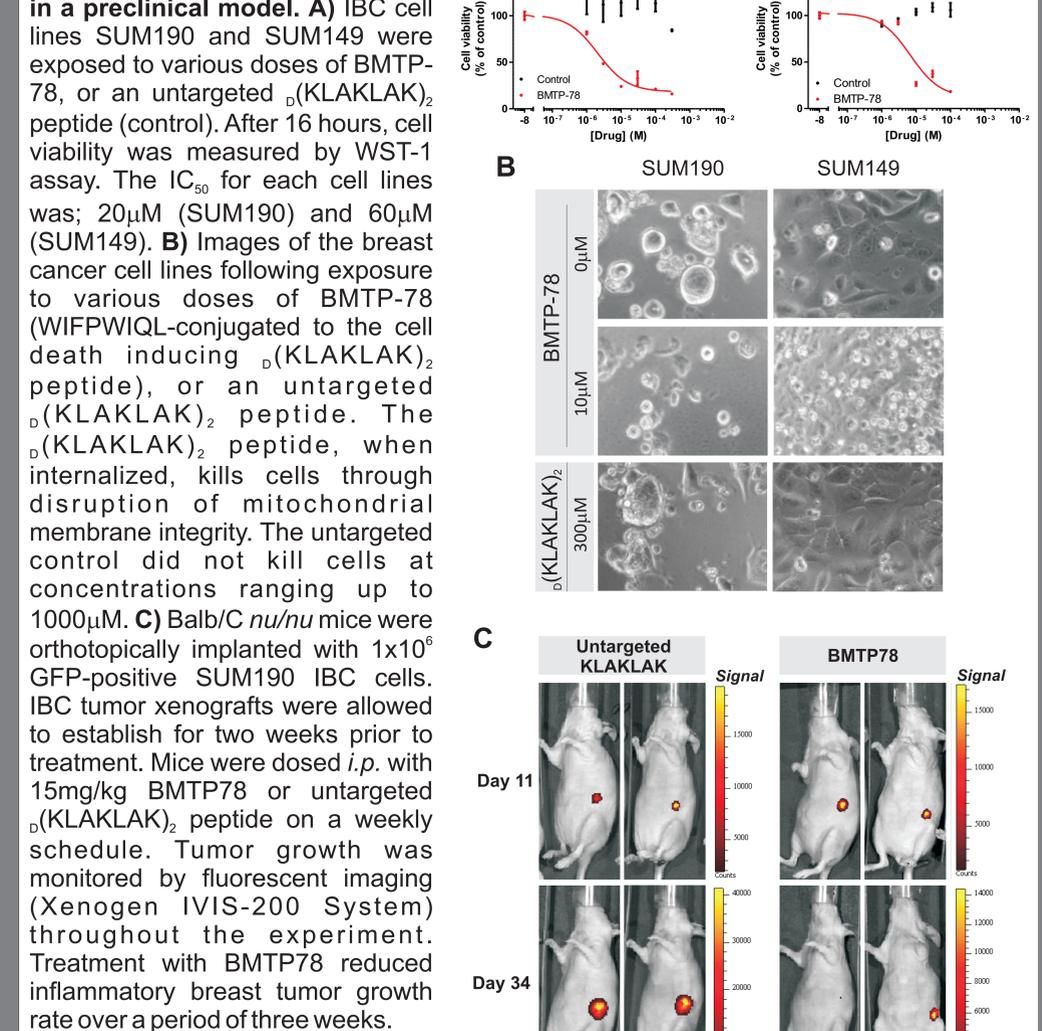


Figure 6. BMT-78 kills IBC cells *in vitro* and inhibits tumor growth in a preclinical model. **A)** IBC cell lines SUM190 and SUM149 were exposed to various doses of BMT-78, or an untargeted $\text{D}(\text{KLAKLAK})_2$ peptide (control). After 16 hours, cell viability was measured by WST-1 assay. The IC_{50} for each cell lines was; 20 μM (SUM190) and 60 μM (SUM149). **B)** Images of the breast cancer cell lines following exposure to various doses of BMT-78 (WIFPWIQL-conjugated to the cell death inducing $\text{D}(\text{KLAKLAK})_2$ peptide), or an untargeted $\text{D}(\text{KLAKLAK})_2$ peptide. The $\text{D}(\text{KLAKLAK})_2$ peptide, when internalized, kills cells through disruption of mitochondrial membrane integrity. The untargeted control did not kill cells at concentrations ranging up to 1000 μM . **C)** Balb/C *nu/nu* mice were orthotopically implanted with 1×10^6 GFP-positive SUM190 IBC cells. IBC tumor xenografts were allowed to establish for two weeks prior to treatment. Mice were dosed *i.p.* with 15mg/kg BMT-78 or untargeted $\text{D}(\text{KLAKLAK})_2$ peptide on a weekly schedule. Tumor growth was monitored by fluorescent imaging (Xenogen IVIS-200 System) throughout the experiment. Treatment with BMT-78 reduced inflammatory breast tumor growth rate over a period of three weeks.

CONCLUSIONS

We demonstrate here that inflammatory breast cancers, a highly aggressive form of breast cancer, express high levels of GRP78. We validated the ability to selectively target GRP78 *in vitro* and *in vivo* through peptides that specifically bind to GRP78. By conjugating these peptides to either phage and/or antibody fragments we produced vectors that could mediate homing towards IBC tumor xenografts. Indeed, we demonstrate that these vectors can be utilized as *in vivo* tracers when coupled to NIR-fluorescent moieties. In a therapeutic approach, we coupled a toxic moiety to the WIFPWIQL-peptide creating a compound, called BMT-78, which induced IBC cell killing *in vitro* and prevented the growth of IBC tumor xenografts *in vivo*.

Taken together, our data infers potential clinical utility for the detection and eradication of IBC tumors via a GRP78-targeted mechanism.