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Biocompatible, Biodegradable, and Enzymatic-Cleavable MRI
Contrast Agents for Early Detection of Bone Metastatic Breast
cancer

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14. ABSTRACT This project proposes to design and test a novel peptide-based MRI contrast agent for early detection of bone metastasis from breast cancer. The proposed imaging agent is consist of bone targeting moiety of Asp8 and MRI imaging moiety of DOTA(Gd) with a cathepsin K cleavable peptide spacer. A solid phase peptide synthesis (SPPS) strategy was used to synthesize peptides. Coupling of the peptide onto DOTA followed by Gd complexation was performed to achieve the proposed imaging agent. Non-targeting and CTSK-insensitive controls were similarly prepared. The obtained contrast agents were characterized in terms of bone specificity, enzymatic degradability and biocompatibility. Preliminary testing of contrast enhanced MR imaging at different time points is ongoing in mice with bone metastasis from breast cancer.					
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Introduction:

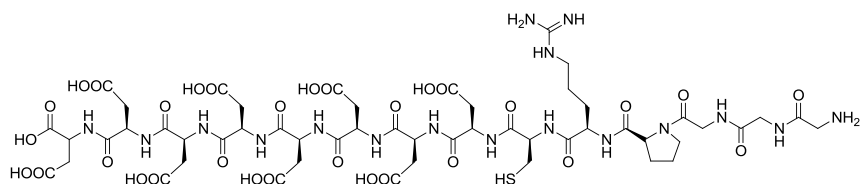
Metastatic breast cancers in bone are very difficult to treat and result in significant morbidity and mortality.(1, 2) Current therapeutic options for breast cancer induced bone-metastases are usually palliative, and planning an individual therapeutic strategy as early as possible to delay skeletal complications is the key to manage patients with bone metastases.(3, 4) Hence, more specific imaging technologies to early detect and accurately diagnose whether and what cancerous bone metastasis is greatly needed in clinical practice.(3) Currently skeletal scintigraphy represents the first line of diagnostic tools in the detection of osseous metastases.(5) However, it suffers from poor sensitivity to lytic bone tumors, which is the case for breast cancer metastases. It also cannot provide structural details of lesions in bone.(5) As a result, MRI is emerging as an alternative imaging modality for bone metastases diagnosis. Contrast enhanced MRI (CE-MRI) assisted with imaging probes can significantly improve early detection of tumor.(6) However, MRI used for the diagnosis of bone metastases in the current clinical setting, has not yet involved the use of contrast agents. Thus, the development of appropriate bone-specific MRI probes for bone metastases is of importance. This proposal addresses this important public health need.

The central hypothesis of this project is that Gd complex in conjugation with Lys-Nle-Pro-Gly-Gly-Asp8 will achieve a novel bone-targeted, biodegradable and enzymatic-cleavable MRI contrast agent. Lys provides an amine group to attach the Gd chelator. Asp8 has a high affinity for bone mineral and has been used as bone-targeting moiety in molecular therapeutics.(7) The use of Asp8 allows active accumulation of contrast agents in bone, thus leading to enhanced MR imaging in skeletal tissues. More importantly, by utilization of a CTSK-sensitive peptide linkage, Gd complex will be released following enzymatic cleavage. This not only reduces potential toxicity, but also leads to dynamic contrast enhancement at bone resorption sites to better detect CTSK activity. As CTSK is suggested as an indicator of bone metastasis from breast cancer,(8) probing CTSK activity at bone turnover sites may suggest the pathogenesis of bone metastasis. In summary, the research proposes a new bone specific contrast agent to explore molecular MR imaging for early detection of bone metastatic breast cancer.

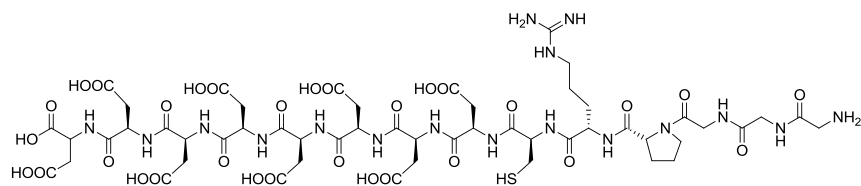
Body

Task 1 was to synthesize and purify peptide-based imaging agents. Proteolysis has been developed as a powerful tool for advanced drug delivery/imaging systems(9, 10). Cathepsin K (CTSK), a protease primarily responsible for bone resorption, has been identified as a valuable therapeutic target for osteoclast-mediated osteolytic disease.(11) Hence, CTSK-cleavable peptides have been explored in the development of molecular imaging probes and drug delivery systems in order to facilitate imaging agents/drugs release in osteolytic microenvironments. Peptides, e.g. RPPG, NPPG and HPPGPQ, have been demonstrated as CTSK-specific substrates in literature.(12-15) However, it is unclear which peptide sequence

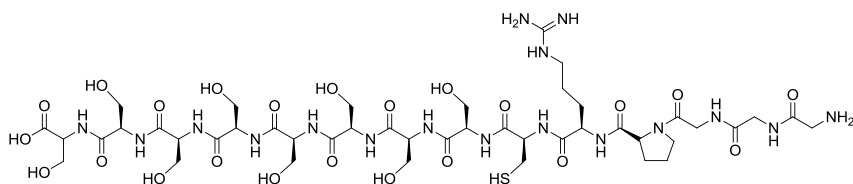
is more sensitive regarding to CTSK cleavage. To address this issue and identify best possible CTSK-cleavable linker for our project, we have synthesized CTSK-activatable imaging agents based on fluorescence resonance energy transfer (FRET) mechanism. We found that upon CTSK treatment, fluorescence intensity increased up to 8.2, 2.5, and 7.6 fold in the case of using RPGG, NPGG and HPGGPQ as substrate, respectively. This study confirmed literatures' results that all of above mentioned peptides are more or less sensitive to CTSK treatment, making them suitable as CTSK-labile peptide for drug delivery or molecular imaging applications.(12-15) Originally we proposed to use NPGG as CTSK substrate. Based on our preliminary results, however, we made appropriate adjustment to switch NPGG to RPGG as the alternative, and use HPGGPQ as the backup option. For this project, we have obtained 4 peptide which all were successfully synthesized by solid phase peptide synthesis (SPPS) strategy, and purified by preparative HPLC. The chemical structures of peptides were shown below. Peptides reacted with DOTA-NHS followed by complexing with Gd(OAc)₃, and then afforded proposed peptide-based MRI contrast agents.



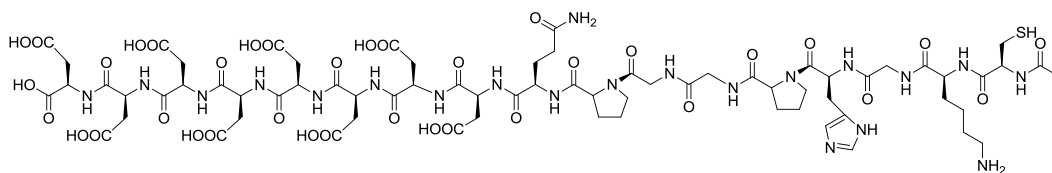
Peptide 1: Asp₈CRPGGG-NH₂



Peptide 2: Asp₈C-rp-GGG-NH₂



Peptide 3: Ser₈CRPGGG-NH₂



Peptide 4: Asp₈-QPGGPHGKC

Task 2 was to characterize peptide-based the contrast agents for the biophysical and biological properties. It was of importance for such agents to possess skeletal binding

affinity, enzymatic degradability and biocompatibility. We confirmed that all of agents with Asp8 residue (bone binding moiety) indeed possess high HA binding affinity. In contrast, the control peptide with Ser8 residue only showed non-specific binding onto HA. In addition, we performed MTT assay in MDA-MB-231 (a breast cancer cell line). The results showed that none of agents indicated cytotoxicity to cells at concentration up to 1000 µg/mL, suggesting peptide-based imaging agents are safe and biocompatible as expected.

Task 3 is to investigate contrast enhanced MR imaging in mice. For this, the proposed imaging agent and also controls will be tested in mice with bone metastasis from breast cancer. The methods to establish animal model of bone metastasis from breast cancer are documented in literature.⁽¹⁶⁾ (Rosol TJ, Tannehill-Gregg SH, LeRoy BE, Mandl S, Contag CH. **Animal models of bone metastasis.** *Cancer*, 97(3), 748-757 (2003)). We have submitted the IACUC protocol for this project, which was approved on December 15, 2010 and will expire until December 14, 2013. Right now we have difficulties to establish enough mice with breast cancer induced bone metastases to test our constructs. Non-cost extension of the research project (04/01/2012 to 03/31/2013) was approved which allows us enough time to perform studies in Task 3.

Key Research Accomplishments

- 4 peptide-based imaging agents were successfully obtained.
- Preliminary test of imaging agents demonstrated that they are biodegradable, biocompatible and safe to use in animals.
- IACUC protocol was approved.

Reportable Outcomes

None at this time

Conclusion

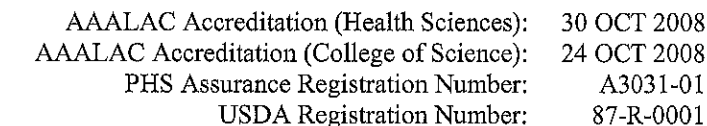
This project proposes to design and test a novel peptide-based MRI contrast agent for early detection of bone metastasis from breast cancer. We have successfully obtained 4 peptides by using a solid phase peptide synthesis (SPPS) strategy. Coupling of the peptide onto DOTA followed by Gd complexation was performed to achieve the proposed imaging agents. Non-targeting and CTSK-insensitive controls were similarly prepared. The obtained contrast agents were characterized in terms of bone specificity, enzymatic degradability and biocompatibility. Preliminary testing of contrast enhanced MR imaging at different time points is ongoing in mice with bone metastasis from breast cancer.

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Appendices

IACUC approval Form



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