Development of a Bone Marrow Targeting Cell Therapy

Thomas Kean*, Lori Duesler*, Tambet Teesalu, Erkki Ruoslahti**, James E Dennis***

*Case Western Reserve University, Cleveland, Ohio, ** Burnham Institute for Medical Research at University of California, Santa Barbara, California

Introduction

One of the major obstacles in stem cell therapies is poor cell engraftment. To address this issue, concurrent investigations were made to develop novel peptides targeting bone marrow and to develop a method to transiently 'paint' targeting molecules onto cells[1]. Painting was studied using a model peptide which had previously been identified as binding to injured tissue[2]. Palmitation was used to enable membrane integration of the peptide. In order to track this peptide, it was modified with a labeled spacer constructed with two things in mind: hydrophilicity and maintenance of peptide activity. The peptide spacer incorporates the hydrophilic amino acids (to space the peptide away from the membrane) preceding the phage-developed targeting sequence and the biotin label allows efficient detection whilst having little effect on the targeting peptide's ligand affinity. Novel bone marrow targeting peptides were sought using *in vivo* phage display in a locally irradiated mouse model that has an internal control (untreated leg). The targeting to irradiated marrow provides an additional level of targeting specificity for bone marrow transplantation applications.

1) Finding peptide candidates using Phage Display

- T7 phage are genetically engineered to express a peptide on its capsid (Fig. 1); 7 amino acids gives a phage pool with 1.28×10^9 different peptides (phage library)
- The phage library was then screened against the bone marrow of a low dose (250 Rads) isolaterally irradiated mouse (Fig. 2)
- After five iterations, phage DNA is sequenced and the peptide sequence determined

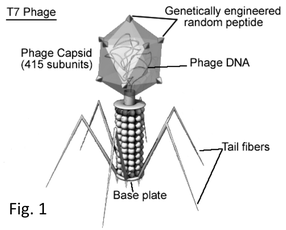

Fig. 1

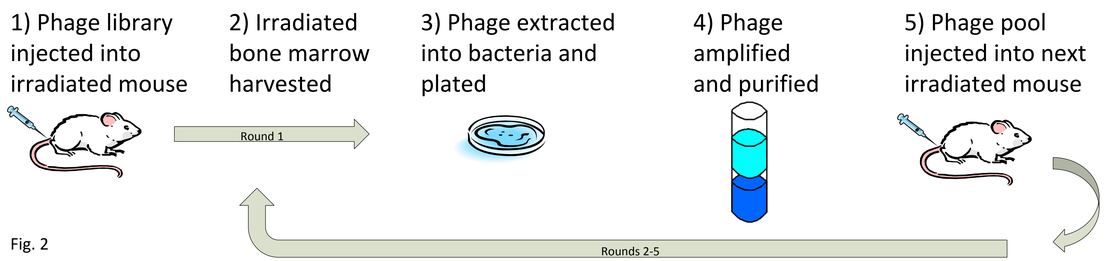

Fig. 2

After 5 rounds of selection, phage were sequenced and most peptides were found to be specific to each leg with little crossover in the lung (Table 1). Phage were then grown individually and animal distribution assessed. Leg titer was greater than lung titer in all of the phage assessed (Fig. 3). All phage, except A2, had greater titer in the irradiated leg than the control, untreated leg. In addition, when tested in a whole body irradiated mouse, spleen uptake of phage A4 was 8-fold higher. This could indicate a preference for the hematopoietic niche.

Table 1: Plaque Sequence Analysis

	Tissue (number of plaques)		
Phage	Untreated Leg	Irradiated Leg	Lung
A1		4	1
A2		3	
A3		2	
A4-9		1	
B1	1	5	
B2-3	4		
B4	2		
B5-11	1		
C1-2	1		1
C3-19			1

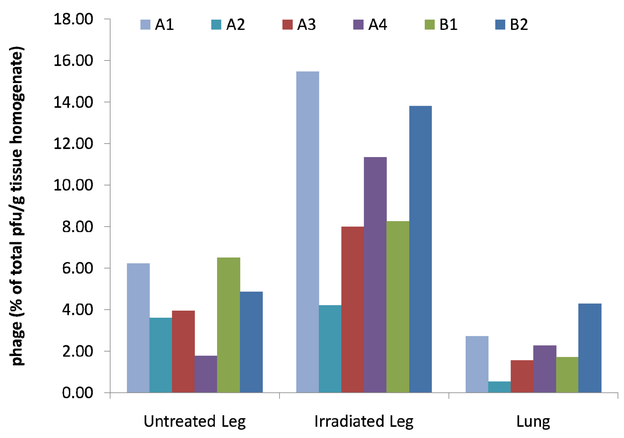

Fig. 3 - Assessment of Phage Localisation in Bone Marrow and Lung
Individual phage identified in the screen (Table 1) were investigated and their titer in the irradiated bone marrow, untreated bone marrow and lung is compared.

2) Assessment of peptide painting

1) Palmitated biotin labeled peptide (PA-CARBio) was tested for its ability to coat mesenchymal stem cells after trypsinisation (Fig. 4). After coating, cells were washed, centrifuged and resuspended in streptavidin-phycoerythrin, then washed and fixed in formalin.

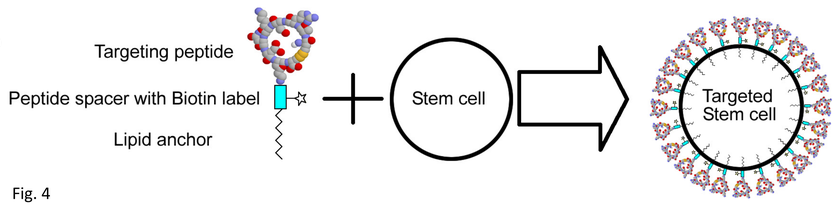

Fig. 4

2) Peptide incorporation was studied using flow cytometry and fluorescence microscopy.

Increasing concentrations of PA-CARBio were assessed by flow cytometry (Fig. 5A) showing increasing cell-associated fluorescence with no plateau. Fluorescence at the lowest (0.5 µg/ml) concentration equates to >20,000 fluorophores per cell, at the highest concentration (50 µg/ml) it is equivalent to over 2 million fluorophores per cell.

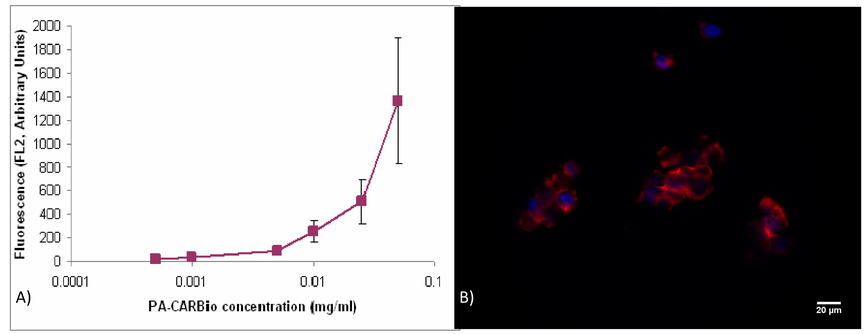

Fig. 5 - Assessment of Palmitated Peptide Uptake
The incorporation of palmitated peptide was assessed using flow cytometry (A) and fluorescence microscopy (B), blue (Hoechst nuclear stain) red (streptavidin-phycoerythrin)

On the right (Fig. 5B) an epifluorescent image of mesenchymal stem cells shows the distribution of the coating. Cytotoxicity of PA-CARBio was assessed using the MTT assay[3], no reduction in cell viability was observed after a 10 minute or 1 hour to the palmitated peptide at any concentration tested (0.5 µg/ml – 0.1 mg/ml)

Conclusions and Future Perspectives

Several interesting peptides have been identified through phage display that have the potential to direct cells to bone marrow (both irradiated and non-irradiated). Palmitation has been shown to enable rapid and efficient peptide incorporation onto the cell surface, and the biotin label provided an effective means of tracking the peptide. The next step is to combine these two technologies and investigate the effect on stem cell migration to bone marrow.

References

1) Dennis et al., J. Orthop. Res., 2004; 2) Järvinen and Ruoslahti, Am. J. Path., 2007; 3) Kean et al., J. Control. Release, 2005

Acknowledgements

We thank Karrie Trevarthen and Simone Edelheit for expert sequencing. This work was supported by grant AR49785 from the NIH and funding from Cell Targeting Inc.