BONE TROPIC PEPTIDES FROM A PHAGE DISPLAY LIBRARY BIND TO MESENCHYMAL CELLS AND POTENTIATE BONE REPAIR

*Madhu, V; *Beck, G; *Huang, D; +*Balian, G
Orthopaedic Research Laboratories, Department of Orthopaedic Surgery* and Department of Biochemistry & Molecular Genetics+, University of Virginia, Charlottesville VA 22908
gb@virginia.edu

Introduction: Significant progress has been made in attempts to modulate bone regeneration by controlling cytokines and growth factors; nevertheless, treatment of osseous defects and fracture non-unions is still a challenge. The objective of this study is to establish if bone tropic peptides isolated from a phage display library can promote in bone defects and to identify the molecular targets in order to understand the mechanism of peptide mediated bone regeneration.

Materials and Methods: Peptide Synthesis. Peptides were selected from a Ph.D 12-mer phage display library kit (New England Biolabs, Beverly, MA) by in vivo biopanning. The unique peptide sequences eluted from bone and marrow were then identified by sequencing phage DNA. Based on initial studies we have selected two peptides designated R1 and L7 for further investigation. Cell staining. Biotinylated peptides were synthesized for the localization of peptide binding to mesenchymal cells. Fluorescein-isothiocyanate (FITC)-labeled avidin was used to localize the binding of peptides on cells grown in vitro by epiluminescence microscopy. Microarray: A cDNA microarray analysis was conducted to examine gene expression profiles in R1 peptide treated D1 mesenchymal cells compared to a control using the Affymetrix GeneChip system. Affinity Chromatography. To identify the peptide targets, cells were lysed with DTT lysis buffer, and the cell lysates were applied to avidin-agarose. The bound proteins were eluted with glycine-HCl buffer, and the eluate analyzed by SDS-PAGE. Surgery procedure: All animal procedures were approved by the Animal Care and Use Committee. Peptides were initially tested in 3 mm unicortical defects. To determine the osteogenic effect of peptides in a critical size defect a gelfoam cylinder (8x3x3 mm3) was soaked in either L7 or R1 (20 mM) solution or in PBS only (control) and placed in the unicortical defect (8x3x3 mm3) created bilaterally on the antero-medial aspect of the tibia of 9-month Fischer 344 rats. The tibias were harvested at 3, 5 and 12 weeks after surgery, fixed in 10% formalin, decalcified in rapid decalcifier for 2 weeks and embedded in paraffin. Serial 10 μm sections were stained with H&E, examined microscopically and analyzed histomorphometrically.

Results: Biotin-labeled R1 was shown to bind to bone marrow cells in culture (Fig.1). L7 promotes the formation of cell clusters similar to cells that undergo matrix mineralization in vitro. Results from microarray showed changes in the expression of several bone related genes shown in Table 1. Visualization of affinity chromatography fractions on SDS-PAGE showed that peptide R1 binds to two components with a molecular mass ~200 kDa and ~ 45 kDa, and that L7 binds three proteins with a molecular mass in the 40-50 kDa range. Histological examination of tibial defects at all time points demonstrated that empty defects without gelfoam or peptide showed no bone repair. In the smaller (3mm) defects, histomorphometry showed that defects treated with peptides contained 2-3 times more bone than gelfoam without peptide. In the larger (8mm) defects, the gelfoam control without peptide showed repair at the edges of the defect but none at the cortex and no bone marrow within the defect site. At 3 weeks, defects with gelfoam+R1 showed extensive cortical bone repair. By 5 weeks, the cortex had thickened and the marrow has begun to be reform. By 12 weeks the defect was completely healed. The thickness and continuity of bone repair in the groups that were treated with R1 peptide was greater than in the control group without peptide. With L7 by 3 weeks the bone forms a single layer of bone at the middle of the defect and a minor amount of gelfoam remained. By contrast to R1, defects treated with L7 showed an abundance of bone with a callus and no bone marrow at 12 weeks (Fig. 2).

Discussion. Our studies demonstrate that peptides R1 and L7 stimulate bone regeneration in vivo. R1 and L7 show distinct properties. R1 binds to marrow mesenchymal cells in vitro and promotes cortical bone and marrow in vivo. By contrast, L7 potentiates bone repair but without marrow regeneration at 12 weeks. Further analysis of the molecular targets and their characterization will demonstrate the mode of action on cells in vitro and tissue regeneration in vivo. Synthetic peptides may have advantages over larger molecules such as speed of preparation, molecular stability, long shelf lives and potentially easier bioengineering applications. These peptides, therefore, may offer attractive alternatives to existing growth factors that stimulate bone regeneration.

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Table 1. Selected microarray result for R1 peptide treatment of mouse D1 cells.

<table>
<thead>
<tr>
<th>Bone related genes</th>
<th>Fold change</th>
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<tbody>
<tr>
<td>Dentin matrix protein 1</td>
<td>+1.62</td>
</tr>
<tr>
<td>Fibronectin 1</td>
<td>+1.52</td>
</tr>
<tr>
<td>Procollagen, type VIII, alpha 1</td>
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<tr>
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<tr>
<td>Syndecan 4</td>
<td>-1.32</td>
</tr>
<tr>
<td>Parathyroid hormone receptor 1</td>
<td>-1.41</td>
</tr>
</tbody>
</table>

Fig. 1 R1 Peptide shows binding to a bone marrow mesenchymal cell (D1).

Fig. 2 Repair tissue in the unicortical defects that were treated with gelfoam+peptide contain more cortical bone at 3, 5 and 12 weeks compared with the controls that were treated with gelfoam alone.

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