Identification and Initial Characterization
of High Affinity Ligands Binding Specifically to Endothelial Progenitor Cells Using Phage Display Screening

A. Veleva[1], C. Patterson[2], S. Cooper[3]
[1] Department of Chemical and Biomolecular Engineering, NCSU, Raleigh, NC,[2] Carolina Vascular Biology Center and Department of Medicine, Department of Pharmacology, and Department of Cell Biology, UNC, Chapel Hill, NC, [3] Department of Chemical and Biomolecular Engineering, OSU, Columbus, OH.

Introduction: The discovery of new peptide ligands that recognize specific cell types promises to be a valuable tool both in research and clinical applications. Cell-type specific peptides can be used as drug delivery vehicles, diagnostic agents, affinity reagents for cell purification, gene therapy delivery agents and research tools to probe the molecular diversity of a cell surface [1]. In this investigation we use cell-specific peptides identified by phage display as a tool to enhance biocompatibility of a biomaterial surface. We performed non-biased selection using an outgrowth of endothelial progenitor whole cells as an affinity matrix. To avoid non specific binding we applied a negative-positive selection approach by pre-incubating the library with non EPC.

Methods: *Bacteriophage panning*: PhD-12 peptide phage display system (New England BioLabs, Beverly, MA) was used for the biopanning experiments. The complexity of the library is in excess of two billion binding clones. To eliminate phage with binding capacity to common receptors the library was pre-adsorbed on human umbilical vein endothelial cells (HUVEC) prior to biopanning on EPC-derived endothelial cells. EPC were isolated from peripheral blood (anticoagulated with 3.8% sodium citrate) using a density gradient centrifugation protocol and subsequently cultured in differentiating medium (EGM-2 and Single Quotes, Clonetics, CA). In the first set of biopanning, the pre-adsorbed phage library was incubated with the EPC outgrowth cell suspension in EGM-2 (1.10^5 cells/ml) for 1hour at 4°C. The unbound phage clones were separated from the cell bound phage complexes by centrifugation. After extensive washing, the unbound phage was eluted and amplified in *E-Coli* culture. An aliquot of the amplified phage ($1.5.10^{11}$ pfu) was subsequently re-applied to newly trypsinized cells for a total of three biopanning rounds and two amplification steps.

Assaying for binding specificity: The specificity of EPC-selected phage clones was determined by biopanning on a panel of other cell types. The biopanning procedure was carried as described above with the exception of including the pre-clearing incubation.

Results / Discussion: We designed a two-step panning strategy to isolate phage that bind specifically to cultured endothelial progenitor cells. First, to decrease non-specific binding we pre-cleared the phage library on non EPC. We incubated the phage library with HUVEC and centrifuged to separate HUVEC-phage complexes and unbound phage clones. Second, the unbound phage pool was incubated with cultured EPC for 1 hour on ice. EPC-bound phage were separated by centrifugation, recovered by bacterial infection, amplified and subjected to two more rounds of selection. To test the selection method, after the third round of biopanning the eluate was titered, and individual phage clones were isolated and amplified. The DNA of randomly chosen phage clones was sequenced and the encoded peptide sequences were deduced. Below we report results from *in-vitro* phage binding assays for representative phage clones. In order to test the specificity of selected phage clones, equal amounts of phage (1×10^{11} pfu) were incubated with different cell lines including HUVEC, HL-60, human neutrophils and lymphocytes. Selected phage clones did not exhibit enhanced binding to any of the other cell types tested, as shown on Figure 1. Of note is the fact that the phage were selected with negative screening procedure only against HUVEC.

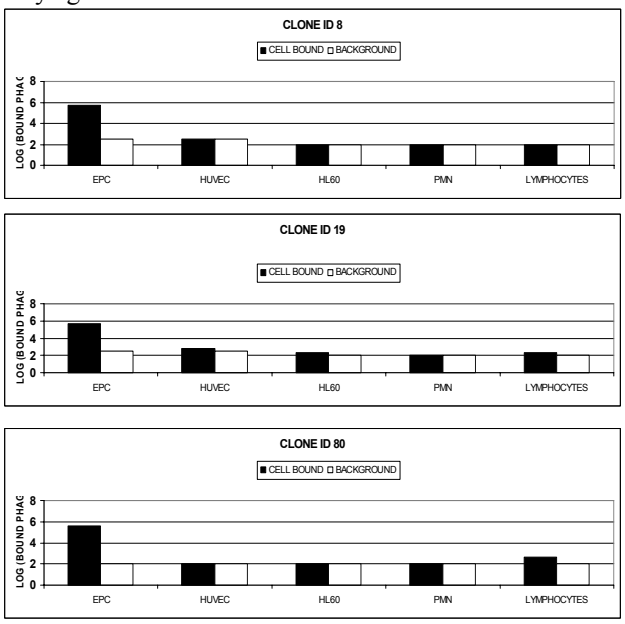

Figure 1: Cell specificity of EPC-selected phage clones. Significant binding was observed to EPC and not to any other cell type tested.

Conclusions: We report the discovery of novel peptides that are highly specific for endothelial progenitor cells (EPC) by screening phage display libraries. Our strategy for isolation of ligand peptides is designed to allow using whole cells as an affinity matrix. We show that binding of phage clones is highly specific for EPC since no binding has been observed on human umbilical vein endothelial cells (HUVEC), HL-60 leukemia cells, human neutrophils and lymphocytes. The results from this study contribute to the development of new strategies to be exploited for the design of biomimetic materials for tissue engineering applications.

References: [1] Rodi DJ and Makowski L., Curr. Opin. Biotechn, 10: 87-93, 1999.