457a Isolation of Tumor Targeting Peptides Using Fluorescent Bacterial Display Libraries

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Cell specific affinity reagents are critical for diagnostic, separation, and therapeutic applications, yet their identification and production remain difficult and time consuming. To address this need, a novel methodology was developed to discover peptide sequences that bind with high specificity to human breast carcinoma cells. *E. coli* expressing both an intracellular fluorescent protein and an outer member protein capable of displaying peptides on the cell surface were used to screen peptide libraries against tumor cells. The peptide variants were genetically fused to an extracellular terminus of a circularly permuted variant of outer membrane protein X. Peptide libraries were designed to be fully random 15mers (X_{15}) or constrained 7mers ($X_2CX_7CX_2$). Selections were performed by co-sedimentation of binding bacteria with tumor cells followed by quantitative fluorescence activated cell sorting which discriminated unlabeled tumor cells from those with bound fluorescent bacteria. Alternating positive selections on tumor cells and negative selections on normal human breast epithelial cells allowed for enrichment of bacterial clones exhibiting specific tumor cell binding, potentially to overexpressed receptors on tumor cells. Further selections toward multiple breast tumor cell lines generated an array of specific peptides that provided a receptor fingerprint, potentially useful for diagnostic applications. The selected fluorescent bacteria resulting from these screens provide self-renewing affinity reagents which allow for single step labeling of target cells for cytometric, microscopic, and array analysis.