mRNA Display Selection Using a Combinatorial 10FnIII Protein Library for Detection and Modulation of Cellular Processes

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Abstract

For years, the natural diversity intrinsic to the mammalian immune system has been harnessed for the generation of specific macromolecular recognition tools. With the development of *in vitro* selection techniques, the ability to create tailor-made, high affinity peptide-based reagents has become more powerful. The directed evolution of peptides and proteins has many applications in proteomics and functional genomics research. Combinatorial peptide libraries based on stable protein scaffolds with diversity contained within defined regions of the domain's surface enable the evolution of novel molecules. One scaffold utilized for *in vitro* selection experiments is the 10^{th} fibronectin type III domain of human fibronectin. This domain is similar to the immunoglobulin fold, although it does not contain disulfides and therefore may be more appropriate for intracellular expression. We have created a new combinatorial library based on this domain and have determined that it is able to tolerate diversity within two loops. Our structured fibronectin library was used for selecting novel, high-affinity reagents by mRNA display. We applied this library towards two important systems, the NF-κB pathway and the SARS coronavirus. In both experiments, we generated high-affinity binders which were functional both *in vitro* and *in vivo*. A modification-specific, phospho-IκBα-binding fibronectin was selected with an affinity of 18 nM. The phospho-IκBα binder was over 1000-fold specific for the phosphorylated state and was able to inhibit IκBα degradation *in vivo*. High-affinity SARS nucleocapsid-binding fibronectins were also selected which were able to inhibit virus replication by over 1000-fold when expressed in SARS infected cells. Both selections demonstrate the utility of the fibronectin library for generating novel protein affinity reagents.

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