Development of Functional Nucleic Acid Aptamers for Inhibition of Cytokines Activity

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Statement of Purpose: At an event of injury, Macrophages detect foreign matter and secrets cytokines which signal other immune cells as a part of the inflammatory response. Inflammatory responses are guided by cytokines, which are produced by cells, such as macrophages, and act on them in an autocrine manner. Quite often, inflammatory responses result in further damage of the injury site. Biomaterials are needed that can regulate these responses, and we are developing strategies for preparing polymeric materials with ligands capable of modulating cytokine activities. The basic strategy is the development of small-molecule ligands with specific affinities for particular cytokines. When incorporated into a polymeric matrix, these ligands will bind their target proteins and should effectively mitigate the inflammatory response. Nucleic acid aptamers are a potential candidate to perform this task. Aptamers are typically single stranded DNAs consisting of 30 to 70 nucleotides (Rimmelle, 2003). It has been suggested that the possibility to find aptamers for every protein is very high, and because of their variety and complexity of 3D structures and binding regions, Aptamers are capable of binding to molecules and proteins with high affinity and specificity (Wilson et., 1999). Literature has shown that highly-selected aptamers have reduced thrombin activity by half in blood clotting event (Bock et., 1992) Selection of aptamers against certain cytokines could provide sufficient effect on cytokine inhibition. IL-1β has been known to play important roles in cell communications when inflammation occurs. Selection against IL-1β will be performed first, and sequencing of these selected aptamers will reveal the critical regions of sequence necessary for IL-1β binding. These aptamers will then covalently bind with a gel material, and in vitro macrophages response to such material will be observed.

Methods: Double stranded DNA library with random sequence of 40 base-pairs in the center and two designated primer regions on each end was purchased from Integrated DNA Technology. PCR was performed to amplify the pool of DNA library. Agarose gel electrophoresis was then run to determine the presence of DNA and confirm if the size of the DNA matches with the original library. The PCR product was then carried through agarose-avidin bead to make the DNA single stranded. Polyacrylamide gel electrophoresis (PAGE) and isopropanol precipitation were applied to purify the single stranded DNA. Absorbance at 260nm was measured to determine the concentration of the DNA. The DNA was then diluted to a concentration that reflects the desired DNA to IL-1β concentration ratio. IL-1β and DNA were mixed to bind and the mixed solution was injected through a Millipore filter. Because of binding interaction between Millipore filter and proteins in general, the DNA with binding affinity to IL-1β will stay on the filter, and the ones without binding affinity will run through. The filter was then cut down into pieces and soaked in elution buffer containing urea. The buffer now contains the product of the selection, and this DNA is ready for next round of selection. The concentration ratio between DNA and IL-1β increases by two fold for each successive round of selection.

Results / Discussion: Figure 1 demonstrates the PAGE after fourth round of selection (16:1, DNA:IL-1β). Columns 1 to 9 indicate the presence of the DNA aptamers, and shows that they have the same length. In Comparison with the DNA ladder, the size of the aptamer is about 80bp, which corresponds to the size of the original DNA library. UV spectrum readings have indicated sufficient DNA concentration after each round of selection. The concentration of DNA before the fourth round of selection is 3.0 μM. These data have confirmed the binding of DNA with IL-1β, and by increasing ratio of DNA and IL-1β, selection for DNA with higher binding affinity to IL-1β has been achieved.

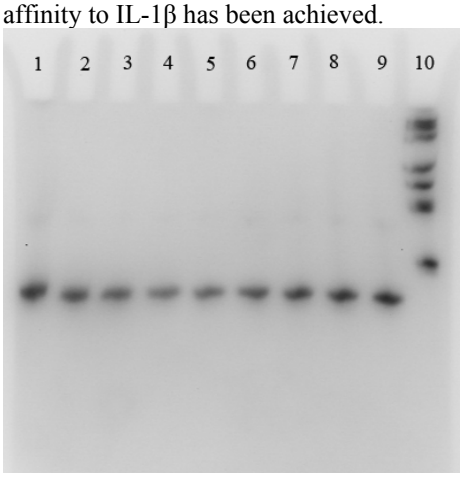

Figure 1. Columns 1to 9 are the aptamers. Column 10 is a DNA ladder with the lowest band indicating 100 bp.

Conclusions: Aptamers with high binding affinity and specificity to IL-1β are being developed. It will carry out until concentration ratio reaches 50 to 1. These aptamers will be designed to covalently attach to a biopolymer, such as hyaluronic acid. We will apply these aptamers to macrophages to test if IL-1β activity is reduced and if the results demonstrate reduction, how much of a reduction will be measured. These ligands will exhibit interference activity against cytokines with wide range of functions.

References:

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