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Track: Pharmaceutical Biotechnology

NUCLEIC ACID APTAMERS AGAINST AGGRECANASES: A NOVEL METHOD FOR DEGENERATIVE DISC DISEASE THERAPY

Yuanyuan Yu and Julian A. Tanner

Department of Biochemistry, the University of Hong Kong, Hong Kong; Email: yuyy@hku.hk

Aptamers are short, single-stranded oligonucleotides, which bind to their targets through 3D conformational complementarity. Aptamers are frequently called 'chemical antibodies' because of their high specificity and affinity. More than 20 aptamers for the treatment of various diseases are evaluated in clinical trials. Role of intervertebral disc is to absorb shock and transmit load, allowing the spine to bend and move. Disc degeneration may cause serious low back pain and affect daily life. Aggrecanases ADAMTS-4 and -5 are critical proteins involved in the development of degenerative disc disease (DDD) and osteoarthritis. They have been used as targets for inhibitor selection against DDD in recent studies both *in vitro* and *in silico*. Small molecules targeted on catalytic domain of aggrecanases have been developed but have broad inhibitory activity which may cause serious side effects. Nucleic acid aptamers can potentially solve this problem. Recent studies on aggrecanases have also been restrained by the low expression level and low solubility of the catalytic domain. Our studies have developed new ways to express, refold and purify human ADAMTS-4 and ADAMTS-5. ADAMTS-4 and ADAMTS-5 catalytic domain incorporating with disintegrin domain and thrombospondin motif are expressed and purified from *E. coli* with high yield for the first time. Specific aptamers will then be tailor selected against each aggrecanase through a process called Systematic Evolution of Ligands by Exponential enrichment (SELEX). Selected aptamers will then be characterized and evaluated as a foundation for further DDD therapeutic development.

Keywords: *Aptamers, degenerative disc disease, ADAMTS-4, ADAMTS-5, SELEX.*

PO-22

Track: Medical Biotechnology

MULTIPLE NON-CANONICAL AMINO ACID INCORPORATION FOR THE SITE-SPECIFIC AND SYNERGISTIC MODIFICATION IN A SINGLE PROTEIN

Kanagavel Deepankumar, Saravanan Prabhu Nadarajan, Hyungdon Yun

School of Biotechnology, Yeungnam University, South Korea; E-mail: hyungdon@ynu.ac.kr

Incorporating non canonical amino acids (NCAAs) into proteins is a powerful *in vivo* methodology that has been increasingly used. Currently, a number of NCAAs was incorporated into protein with the help the methodology such as reassignment of sense (residue specific incorporation) and non sense codon (site specific incorporation). Both methods balance one another in many ways. However, these two methods have a similar drawback in that they allow only incorporation of a single NCAA into the recombinant protein. Our group has developed an easy method to introduce two NCAAs containing different chemical moieties by coupling residue-specific and site-specific incorporation methods in a single protein. So far, all the techniques have demonstrated the possibility of introducing MNCAAs into a single protein; however, no studies have effectively utilized a multifunctional single protein in an effective way to show potential applications. Here, we used the MNCAA incorporation technique for self oriented immobilization (site- specific), to improve protein functionality, and for protein labeling (residue specific). For the purpose, we selected the surface exposed N-terminal residue (Lys15) of the green fluorescent protein (GFP) as a model protein. We introduced an amber codon (Lys15TAG) for the site-specific incorporation of L-DOPA using an evolved Methanococcus jannaschii tRNA/synthetase pairs and simultaneously (2S, 4S)-4-fluoroproline (4S-FPro) was selected for residue specific incorporation. In the next experiment, we prepared the MNCAAs protein with the methionine (Met) surrogate L-homopropargylglycine (L-HPG) (residue-specific incorporation) along with L-DOPA (site-specific incorporation) in GFP (GFPdphpg). The site-specific incorporation of L-DOPA into the protein will allow the protein to be immobilized in a controlled manner through Michael addition, the (2S, 4S)-4-fluoroproline (4S-F-Pro) was selected to improve