"Development of DNA Aptamers Against Growth Hormone-Releasing Peptides for Detection in Serum and Mass Spectral Analyses"

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PROJECT REVIEW

Under this project, Operational Technologies Corporation will develop high affinity and highly specific DNA aptamers to bind the GH-releasing peptides GHRP-6, Ipamorelin and the GHRP-2 main metabolite AA-3 (D-Ala-D-(beta-naphthyl)-Ala-Ala-OH). The targets will be covalently attached to magnetic microbeads (MBs) to select for the highest affinity DNA aptamer candidates in commercially available certified disease-free human serum and urine to ensure highly specific aptamers for their cognate targets in real samples. Following 8-10 rounds of aptamer selection and PCR amplification, aptamer candidates will be cloned and sequenced. DNA sequences will be analyzed for partial and full-length consensus sequences. All candidate aptamers will also be screened by ELISA-like (ELASA) colorimetric microplate assays to rank their relative affinities and specificity for their intended targets as well as related and unrelated targets. The top candidate aptamers from ELASA screening will also be characterized by Surface Plasmon Resonance (SPR) analyses to determine Ka/Kd values versus their intended targets.

The top aptamer candidates will then be attached to MBs and used to probe spiked human serum and urine samples for the three different GHRP or metabolite targets. Validation of aptamer-MB pulled down methods will be accomplished by mass spectral analysis at the core proteomics laboratory of the University of Texas Health Sciences Center in San Antonio, TX. The aptamer-MB pull down protocol will be optimized to include aptamer-MB concentration, capture and elution times and chemical conditions such as pH, ionic strength and the addition of various detergents or other potential additives. Successful development of aptamer-MB pull-down methods may enhance WADA's ability to concentrate GHRPs or their metabolites from body fluids and enhance mass spectral GHRP detection capabilities.

Results and Conclusions:

Although not entirely successful for each of the 3 GHRPs, the basic requirements of this grant have been fulfilled in that several aptamers were developed in a 1:10 diluted human serum or undiluted human urine environment. Most of the 6 lead aptamers were subsequently shown to at least somewhat pull down their cognate target GHRPs in whole human serum or urine with successful detection by ESI-TOF mass spectrometry (MS). In some cases, detection by MS following aptamer-MB pull down and acid-elution failed, but could be corrected by adding 5-fold more aptamer-MBs. In another case, the pull down failure may be due to binding of the G6U-18R to a higher MW (45-60 kD) protein which interfered or competed with binding of the spiked G6 peptide. This same G6U-18R aptamer did, however, detect G6

in urine by MS as it was developed to do. The negative controls lacking aptamers on SAv-MBs demonstrated that the pull down assays were dependent on the specific aptamers tethered to the MBs and that non-specific binding of the GHRPs to the SAv-MBs was essentially non-existent.

It is noteworthy, that with the exception of the 45-60 kD protein pulled down in serum by the two lead anti-G6 aptamer candidates, the Coomassie Blue-stained electrophoresis gels of the pull down acid-eluates are clear. This observation suggests very specific and high affinity binding by the aptamers to their cognate GHRPs because very little else was pulled down on the surface of the aptamer-MBs in serum or urine. Of course, these aptamers were intentionally selected in 10% pooled serum and 100% pooled urine to minimize non-specific binding of the final selected lead aptamers. It is rather surprising that these 6 lead aptamers bound their cognate GHRP targets with low ng detection limits in buffer by ELASA, but failed to bind in serum and urine matrices by ELASA. Fortunately, these same aptamers on MBs were mostly able to detect their peptide targets in serum and urine by MS. This suggests that: 1) MS is more sensitive than ELASA and/or 2) active probing of a liquid sample using mobile aptamer-MBs binds more target than a static well surface. Taken together, albeit not perfect, the use of aptamers on MBs for pull down, purification or concentration of target peptides in serum or urine is a promising technique especially when coupled to MS detection which could significantly aid WADA in its search for doping athletes.