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Multi-Functional Superparamagnetic Iron Oxide Particles as Cancer Therapeutic Agents

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Superparamagnetic iron-oxide nanoparticles (SPIONs) are magnetic nanoparticles may be useful for early detection and treatment of cancers. SPIONs provide therapeutic drug-loading capabilities and targeting specificity through the use of antibodies or receptor specific tags. These versatile particles are prime candidates for improving current means to the treatment of cancer and potentially other diseases. This multi-disciplinary project includes various milestones and multiple aims: i) identify and optimize SPION design parameters that optimize aqueous stability and maximize amphiphilic character, ii) evaluate SPION drug storage and release characteristics, and iii) demonstrate binding and entry into targeted cells. The specific aim of this project is the coupling of cell-specific targeting agents to SPIONs. An antisense oligonucleotide against Survivin mRNA was synthesized by solid-phase DNA synthesis with an amino-terminated linker on the 3' end (5'CCCAGCCTTCCAGCTCCTTG-(CH2)6-3'NH2). SPIONs were prepared and then coated with a copolymer containing surface carboxylic acid groups (-COOH). The carboxylic acid groups were activated by treatment with N-((3-dimethylamino)-propyl)-Nethyl carbodiimide and coupled to oligonucleotides via the –NH2 terminus to the –COOH groups resulting in the formation of an amide linkage between the –COOH groups of the SPION and the –NH2 of the antisense oligonucleotide. Circular dichroism (CD) studies were performed to quantify/optimize coupling and to demonstrate antisense Survivin duplex formation was not inhibited by the presence of the SPION. CD results were correlated with agarose gel electrophoresis data and demonstrated oligonucleotides coupling to the SPION and that the SPION did not significantly alter duplex formation. Future studies will target cellular absorption and antisense binding to Survivin mRNA using confocal microscopy (Supported, in part, from NIH GM081348 grant and the WVU Research Corporation).

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Ultrahigh-Performance Nano LC-MS/MS Analysis of Complex Proteomic Samples

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Determination of the proteome and identification of biomarkers are required to monitor dynamic changes in living organisms and predict the onset of an illness. One popular method to tackle contemporary proteomic samples is called shotgun proteomics, in which proteins are digested, the resulting peptides are separated by high-performance liquid chromatography (HPLC), and identification is performed with tandem mass spectrometry. Digestion of proteins typically leads to a very large number of peptides. For example, digestion of a cell lysate easily generates 500,000 peptides. The separation of these highly complex peptide samples is one of the major challenges in analytical chemistry. The main strategy to improve the efficiency of packed columns is either to increase column length or to decrease the size of the stationary phase particles. However, to operate these columns effectively, the LC conditions need to be adjusted accordingly. Naturally, the on-line coupling to MS systems has to be taken into account in the optimization process. Here, the authors report on the performance of nano LC columns operating at ultrahigh pressure. The effects of column parameters (particle size and column length) and LC conditions (gradient time, flow rate, column temperature) were investigated with reversed-phase (RP) gradient nano LC. High-resolution LC-MS separations of complex proteomic peptide samples are demonstrated by combining long columns with 2 μm particles and long gradients. The effects of LC parameters on performance and the influence on peptide identification are discussed.

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Comprehensive Biomarker Discovery Platform Reveals Qualified MRM Assay for Prediction of INF/Ribavirin Treatment Response in Hepatitis C Patients

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The current standard of care for chronic Hepatitis C (CHC) results in sustained viral response in only half of patients and also has significant side effects. Improved pre-treatment clinical predictors of response are needed to individualize treatment and select individuals most appropriate for new therapy. We have identified 10 peptides to 6 proteins which can differentiate non-responders from responders with high accuracy based on pre-treatment samples. Serum samples were randomized, immunodepleted (MARS 14), and digested with trypsin prior to LC-MSE or LC-MRM analysis. LC-MSE analyses were performed on a Waters nanoAcquity LC and QToF Premier, while MRM analyses were performed on nanoAcquity and Xevo TQ. Rosetta Elucidator® was used to quantify all LC-MSE data. PLGS v2.4 was used to make peptide identifications. MRM method generation and sample quantitation was performed with Skyline v0.6. The response signature was initially identified from the analysis of 96 samples (Duke Hepatology Clinical Research database) by LC-MSE on a QToF mass spectrometer, using sparse latent factor regression analysis. This yielded a group of almost 400 candidate peptides which were part of 3 metaprotein predictors; these peptides were then curated based on set selection criteria to give 86 target peptides for MRM analysis. MRM analysis of the original 96 samples yielded a final group of 10 peptides which maintained Bonferroni-corrected statistical significance for predicting treatment response. The assay has been qualified by a blinded analysis of an all comers clinical trial sample set (PEDS-C, NIH, n=51) using the MRM method, which yielded an AUROC of 0.91 with a sensitivity of 0.828 and specificity of 0.786. The analysis of an additional CHC clinical trial cohort (Chariot, n = 243) is ongoing. This presentation will utilize CHC treatment response as a case study on the critical analytical, statistical, and