Shotgun proteomics

- Cerebrospinal fluid protein biomarkers are associated with response to multiagent intraventricular chemotherapy in patients with CNS lymphoma
- A Shotgun Proteomic-Based Approach with a Q-Exactive Hybrid Quadrupole-Orbitrap High-Resolution Mass Spectrometer for the Assessment of Pesticide Mixture-Induced Neurotoxicity on a 3D-Developed Neurospheroid Model from Human Brain Meningiomas: Identification of Trityl-Post-Translational Modification
- Establishing the utility of multi-platform liquid biopsy by integrating the CSF methylome and proteome in CNS tumours
- Shotgun Proteomic-Based Approach with a Q-Exactive Hybrid Quadrupole-Orbitrap High-Resolution Mass Spectrometer for Protein Adductomics on a 3D Human Brain Tumor Neurospheroid Culture Model: The Identification of Adduct Formation in Calmodulin-Dependent Protein Kinase-2 and Annexin-A1 Induced by Pesticide Mixture
- Proteomics evaluation of five economical commercial abundant protein depletion kits for enrichment of diseases-specific biomarkers from blood serum
- Leveraging the CSF proteome toward minimally-invasive diagnostics surveillance of brain malignancies
- Glioblastoma CUSA Fluid Protein Profiling: A Comparative Investigation of the Core and Peripheral Tumor Zones
- Increased CSF Levels of Apolipoproteins and Complement Factors in Trigeminal Neuralgia Patients-In Depth Proteomic Analysis Using Mass Spectrometry

Shotgun proteomics is a high-throughput approach used to identify and quantify proteins in complex biological samples. It involves the digestion of proteins into peptides, followed by their separation and analysis using mass spectrometry. The term “shotgun” refers to the random fragmentation of proteins into peptides without prior separation of individual proteins.

Here's an overview of the key steps involved in shotgun proteomics:

Protein extraction: Proteins are extracted from the biological sample of interest. The sample can be cells, tissues, biofluids (such as blood or urine), or any other protein-containing material.

Protein digestion: The extracted proteins are enzymatically digested into smaller peptide fragments. Trypsin is the most commonly used protease for this purpose, as it specifically cleaves proteins at the carboxyl side of arginine and lysine residues, generating peptides.

Peptide separation: The resulting mixture of peptides is usually complex, so separation techniques are employed to reduce sample complexity and enhance detection sensitivity. Liquid chromatography (LC) is commonly used to separate peptides based on their physicochemical properties such as hydrophobicity, charge, or size.

Mass spectrometry (MS) analysis: The separated peptides are introduced into a mass spectrometer for identification and quantification. MS allows for the measurement of the mass-to-charge ratio (m/z) of ions generated from the peptides. Tandem mass spectrometry (MS/MS) is often employed, where peptides are fragmented, and the resulting fragment ions are analyzed to determine their amino acid sequence.

Database searching and protein identification: The acquired mass spectra are compared against protein databases using bioinformatics tools. This process, known as database searching, matches the

observed spectra to theoretical spectra generated from known protein sequences. By identifying peptides and matching them to specific proteins, the proteins present in the original sample can be identified.

Protein quantification: Shotgun proteomics can be used for relative or absolute protein quantification. Relative quantification compares protein abundance between different samples, while absolute quantification determines the actual concentration of proteins. Various methods can be employed, such as label-based approaches (e.g., stable isotope labeling) or label-free methods (e.g., spectral counting or intensity-based measurements).

Data analysis: Once protein identification and quantification are obtained, bioinformatics tools are used to analyze the data. This can involve statistical analysis, functional annotation, pathway analysis, and visualization to gain insights into the biological processes and functions associated with the identified proteins.

Shotgun proteomics is a powerful tool in various fields of research, including biomarker discovery, systems biology, drug development, and understanding protein dynamics in diseases. It allows for the comprehensive analysis of proteomes and provides valuable information about protein expression, post-translational modifications, and protein-protein interactions within complex biological systems.

Ahsan et al. benchmarked five different serum abundant protein depletion (SAPD) kits with regard to the identification of disease-specific biomarkers in human serum using bottom-up proteomics. As expected, the IgG removal efficiency among the SAPD kits is highly variable, ranging from 70% to 93%. A pairwise comparison of database search results showed a 10%-19% variation in protein identification among the kits. Immunocapturing-based SAPD kits against IgG and albumin outperformed the others in the removal of these two abundant proteins. Conversely, non-antibody-based methods (i.e., kits using ion exchange resins) and kits leveraging a multi-antibody approach were proven to be less efficient in depleting IgG/albumin from samples but led to the highest number of identified peptides. Notably, the results indicate that different cancer biomarkers could be enriched up to 10% depending on the utilized SAPD kit compared with the undepleted sample. Additionally, functional analysis of the bottom-up proteomic results revealed that different SAPD kits enrich distinct disease- and pathway-specific protein sets. Overall, the study emphasizes that a careful selection of the appropriate commercial SAPD kit is crucial for the analysis of disease biomarkers in serum by shotgun proteomics [1)].

CSF samples were retrospectively retrieved from the Penn State Neuroscience Biorepository and profiled using shotgun proteomics. Proteomic signatures were identified using machine learning classifiers and survival analyses.

Using 30 µL CSF volumes, we recovered 755 unique proteins across 73 samples. Proteomic-based classifiers identified malignancy with the area under the receiver operating characteristic (AUROC) of 0.94 and distinguished between tumor entities with AUROC ≥0.95. More clinically relevant triplex classifiers, comprised of just three proteins, distinguished between tumor entities with AUROC of 0.75-0.89. Novel biomarkers were identified, including GAP43, TFF3, and CACNA2D2, and characterized using single-cell RNA sequencing. Survival analyses validated previously implicated prognostic signatures, including blood-brain barrier disruption.

Reliable classification of intra-axial malignancies using low CSF volumes is feasible, allowing for

longitudinal tumor surveillance [2)]

characterize the protein profile of cavitating ultrasound aspirator fluid of newly diagnosed and recurrent glioblastoma comparing diverse zones of collection, i.e., tumor core and tumor periphery, with the aid of 5-aminolevulinic acid fluorescence. The samples were pooled and analyzed in triplicate by LC-MS following the shotgun proteomic approach. The identified proteins were then grouped to disclose elements exclusive and common to the tumor state or tumor zones and submitted to gene ontology classification and pathway overrepresentation analysis. The proteins common to the distinct zones were further investigated by relative quantitation, following a label free approach, to disclose possible differences of expression. Nine proteins, i.e., tubulin 2B chain, CD59, far upstream element-binding, CD44, histone H1.4, caldesmon, osteopontin, tropomyosin chain and metallothionein-2, marked the core of newly diagnosed glioblastoma with respect to tumor periphery. Considering the tumor zone, including the core and the fluorescence-positive periphery, the serine glycine biosynthesis, pentose phosphate, 5-hydroxytryptamine degradation, de novo purine biosynthesis and Huntington disease pathways resulted statistically significantly overrepresented with respect to the human genome of reference. The fluorescence-negative zone shared several protein elements with the tumor zone, possibly indicating the presence of pathological aspects of glioblastoma rather than of normal brain parenchyma. On the other hand, its exclusive protein elements were considered to represent the healthy zone and, accordingly, exhibited no pathways overrepresentation. On contrary to newly diagnosed glioblastoma, pathway overrepresentation was recognized only in the healthy zone of recurrent glioblastoma. The TGFβ signaling pathway, exclusively classified in the fluorescence-negative periphery in newly diagnosed glioblastoma, was instead the exclusive pathway classified in the tumor core of recurrent glioblastoma. These results, preliminary obtained on sample pools, demonstrated the potential of CUSA [3)]

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2)
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