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Materials and Method to In Situ Isolate Phosphoproteins in Extracellular Vesicles and Exosome

Engineered glycoproteins displayed on cell membranes for potent antibody response against Hepatitis C.

Researchers at Purdue have developed an Extracellular Vesicle to phosphoprotein (EVTOP) strategy which benefits chemotherapeutic outcome assessment, disease diagnosis, and disease progression monitoring. This EVTOP strategy uses a dual affinity magnetic probe to directly circulating extracellular vesicles (EVs) from capture cerebrospinal fluid (CSF), plasma, urine, or saliva, and enrich their iphosphopeptides for the phosphoproteomic assessment. This technology utilize the properties of octa-arginine and titanium (IV) to enable efficient isolation of circulating EVs and enhanced identification of signaling molecules that are critical in many diseases.

The researchers were able to demonstrate the EVTOP strategy for analyses of clinical primary central nervous system lymphoma (PCNSL) samples in comparison with controls. This led them to identify 18 phosphoproteins that demonstrated a significant reduction in intensity among at least eight PCNSL patients post-chemotherapy. The identified phosphoproteins include SPP1, TRH, SCG2, TNC, and SELENOP and are known to be associated with neurological diseases. The use of this EVTOP strategy allowed researchers to identify the listed phosphoproteins as biomarkers for evaluating the chemotherapeutic performance in PCNSL patients due to the enriched presence of signaling pathways such as PI3K-Akt, PI3K-Akt-mTOR, and oncogenic MAPK, all of which are related to PCNSL development. This novel technology allows for more efficient and accurate biofluid phosphoproteomics, reduces the required volume of samples, and shortens the time for sample processing.

Technology Validation:

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Pharmaceuticals/Pharmaceutical Packaging & Delivery Systems
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- Enrichment efficiency of EVTOP was tested using synthetic phosphopeptides with known sequences. Results showed the method could recover an average of 84.36% of the synthetic phosphopeptides added in the lysis, and approximately 36.5% of the synthetic phosphopeptides were recovered from cerebrospinal fluid (CSF) extracellular vesicle (EV) samples

- EVTOP method was applied to 21 primary central nervous system lymphoma (PCNSL) patient samples and 21 non-PCNSL control samples for differential phosphoproteomics analysis, leading to the identification of upregulated phosphoproteins in PCNSL samples.

- Parallel reaction monitoring and parallel accumulation-serial fragmentation (prm-PASEF) were used to monitor the intensity changes of these upregulated phosphoproteins before and after chemotherapy in PCNSL patients. This helped to validate identified phosphoproteins as potential biomarkers for evaluating chemotherapeutic performance.

Advantages:

- More efficient isolation and identification process compared to the traditional centrifugation method of circulating extracellular vesicles (EVs) and their phosphopeptides; only microliters of biofluids such as plasma are needed to monitor the change of signaling molecules

- More accurate results due to enriched phosphopeptides

Applications:

- Disease monitoring

- Disease screening

- PCNSL chemotherapeutic outcomes assessment

Publications:

Profiling Phosphoproteome Landscape in Circulating Extracellular Vesicles from Microliters of Biofluids through Functionally Tunable Paramagnetic Separation

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TRL: Biotechnology

Intellectual Property:

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PCT-Patent, 2024-03-06, WO

NATL-Patent, 2025-09-06, United States

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