Evaluation of engineered multi-nanoparticle-based proteomics analysis for unbiased, deep, and rapid analysis of fetal bovine serum derived cell culture media

Other

Bryn Levitan (blevitan@seer.bio), Seer, Inc., **Xiaoyan Zhao**, , **Heather Ha**, , **Margaret Donovan**, Seer, **Ryan Benz**, Seer Inc, **Mathew Ellenberger**, Seer Inc, **Khatereh Motamedchaboki**, Seer Inc, **Asim Siddiqui**, Seer Inc., **Marty Goldberg**, Seer Inc., **Omid Farokhzad**, Seer Inc.

The conditioned media of different cell cultures are widely used for a variety of biological applications in-vitro; including characterization of secreted proteins from different cell types into the media under different conditions or treatments to understand underlying observed biological functions. However, to keep cells viable, a set of abundant proteins in fetal bovine serum (FBS), are included in the media for mammalian cell culturing, and create an extra level of complexity by affecting the dynamic range of protein concentration. This complexity and wide dynamic range are similar to challenges researchers have in plasma proteomics for in-depth proteomics analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS) which necessitates complex workflow and trade-offs between throughput, scalability, coverage, and precision. To solve this challenge, we applied a deep and scalable proteome profiling platform to analyze FBS based HeLa cell media directly on the automated ProteographTM Product Suite. This approach leverages multiple nanoparticles (NPs), engineered with distinct physicochemical properties to provide broad coverage of the complex proteomes at scale.

In this study, we used 250 uL of media harvested from HeLa cells. We applied the media directed to ProteographTM platform and analyzed peptides with data-dependent acquisition (DDA) LC-MS analysis using 2hrs LC gradient per NP per sample. Our results demonstrate detection of more than ~3000 cell-derived proteins in the HeLa cell culture supernatant containing FBS. The ProteographTM platform offered ~10X improvement in coverage comparing to results derived from direct digestion of same media material, enabling identification lower abundant cytokines in the culture media, which are not robustly detected by conventional proteome approaches and only achievable with complex proteomics workflows including depletion and fractionation.

This study evaluates performance of the ProteographTM platform combined with label-free mass spectrometry analysis for deeper profiling secreted proteins in cell culture media in a rapid fashion, enabling deep and unbiased large-scale conditioned media studies to detect novel insights.