High-Throughput Proteomic Insights from Cell Culture Supernatant and Lysates

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Abstract

High-throughput screening using traditional or 3D organoid cell culture has become a foundational tool in the process of identifying compounds, small molecules, or biologics with potential pharmaceutical activity that can be studied further in lead optimization studies. Recent advances in next generation sequencing (NGS) have built upon this research by incorporating low-cost, high-throughput transcriptional screening assays with phenotyping approaches. Incorporating proteomic insights with a multiomic approach provides a more complete understanding of cellular activity. Recent advances in proteomics technologies, including proximity-extension assays, have enabled reduced-cost, high-plex, scalable analysis of the proteome without sacrificing quality.

In this proof of principal study, RNA-Seq and Olink proteomics were used on a series of samples as a means to incorporate high-plex multiomic analysis in the early drug discovery pipeline. HEK293T cells were exposed to varying levels of TNF-alpha. Cell lysates and culture medium were collected and analyzed in parallel to characterize the intra-cellular and inter-cellular signaling responses. To test both the sensitivity, specificity and reproducibility of the proteomics assay, one microliter per sample was used to determine abundance of approximately 1,000 proteins in the well-characterized NF-kB response. Transcriptional changes were also confirmed using traditional RNA-Seq methods. As these results show, high-throughput proteomic analysis, together with transcriptional alterations detected by RNA-Seq, support and confirm multiomic changes, indicating proteomic analysis can also be leveraged for drug screening.

Acknowledgements

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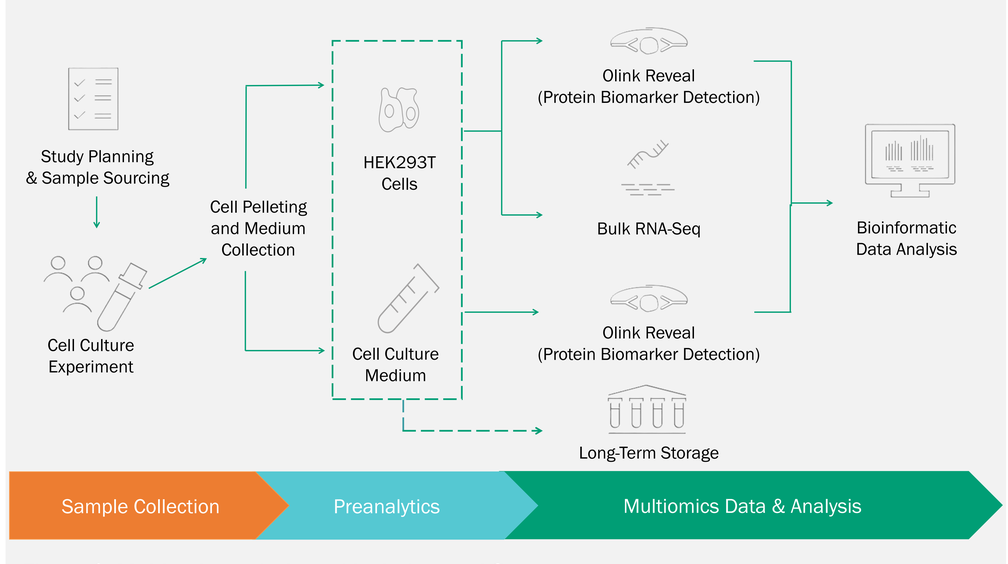

Figure 1. Multiomic analysis and isolation workflow. 16 HEK293T cell culture samples were treated with 0 ng/ml, 1 ng/ml, 10 ng/ml, or 100 ng/ml of TNF-α and incubated for eight hours. Four wells that had only media were incubated with the varying levels of TNF- α as well as a control. Cells were pelleted, supernatant was collected and both cell pellets and supernatant were snap frozen and stored. The cells were lysed and protein and RNA was isolated from each sample and stored at -80˚C. The RNA was processed on non-stranded RNA-Seq with Poly(A) selection. Protein lysate and cell supernatant samples were processed on Olink® Reveal for proteomic biomarker detection.

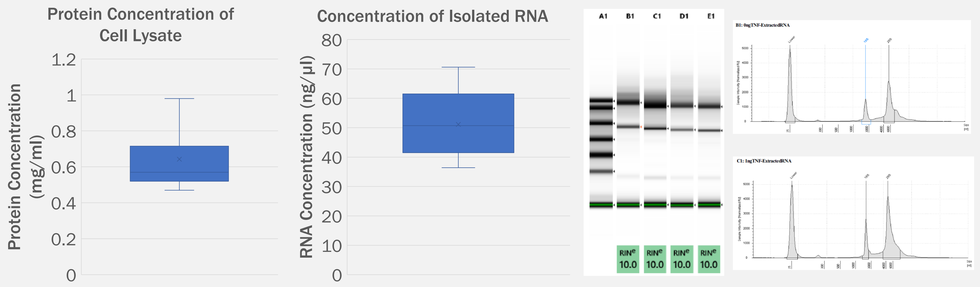

Figure 2. Sample quality. Initial sample quality was evaluated for the lysate and isolated RNA. The targeted protein concentration was 0.5 mg/mL and RNA concentration was targeted for a minimum of 20 ng/µl. The protein concentration was measured by a Bradford assay and RNA concentration was measured by Qubit™. Both protein and RNA consistently met their targets. The RNA was then assessed by RNA ScreenTape for TapeStation® to assess the quality of each sample. All samples scored a RINe of 10, which is the highest possible quality score and indicative of high quality and fully intact RNA.

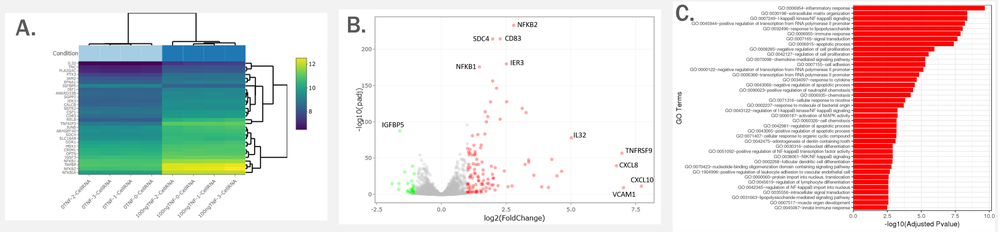

Figure 3. RNA-Seq analysis. The RNA isolated from 16 HEK293T cell culture samples treated with varying levels of TNF-α were prepared using bulk RNA-Seq and sequenced on an Illumina® NovaSeq™ X Plus. (A) A bi-clustering heatmap was used to visualize the expression profile of the top 30 differentially expressed genes (DEGs) after TNF-α treatment. Consistent with literature of this well-characterized pathway, we observed induced expression of *NFKBIA*, *NFKB1*, *NFKB2*, and *RELB* showing activation of the NF-κB pathway in response to TNF-α dosing. (B) The global transcriptional change across the control group and the test group was visualized by a volcano plot where each data point represents a gene where red and green points represent significantly up and down DEGs respectively. Following TNF-α treatment, NF-κB pathway activation is highlighted by increased transcription of markers of inflammation mediators, such as cytokines, chemokines, and adhesion molecules, which collectively contribute to the inflammatory response. (C) Significant DEGs were clustered by their gene ontology, and the gene ontology terms were tested and sorted by their adjusted p-value. Activation of the NF-κB pathway is evidenced by enrichment of the inflammatory response genes and stimulation of the immune defense mechanisms.

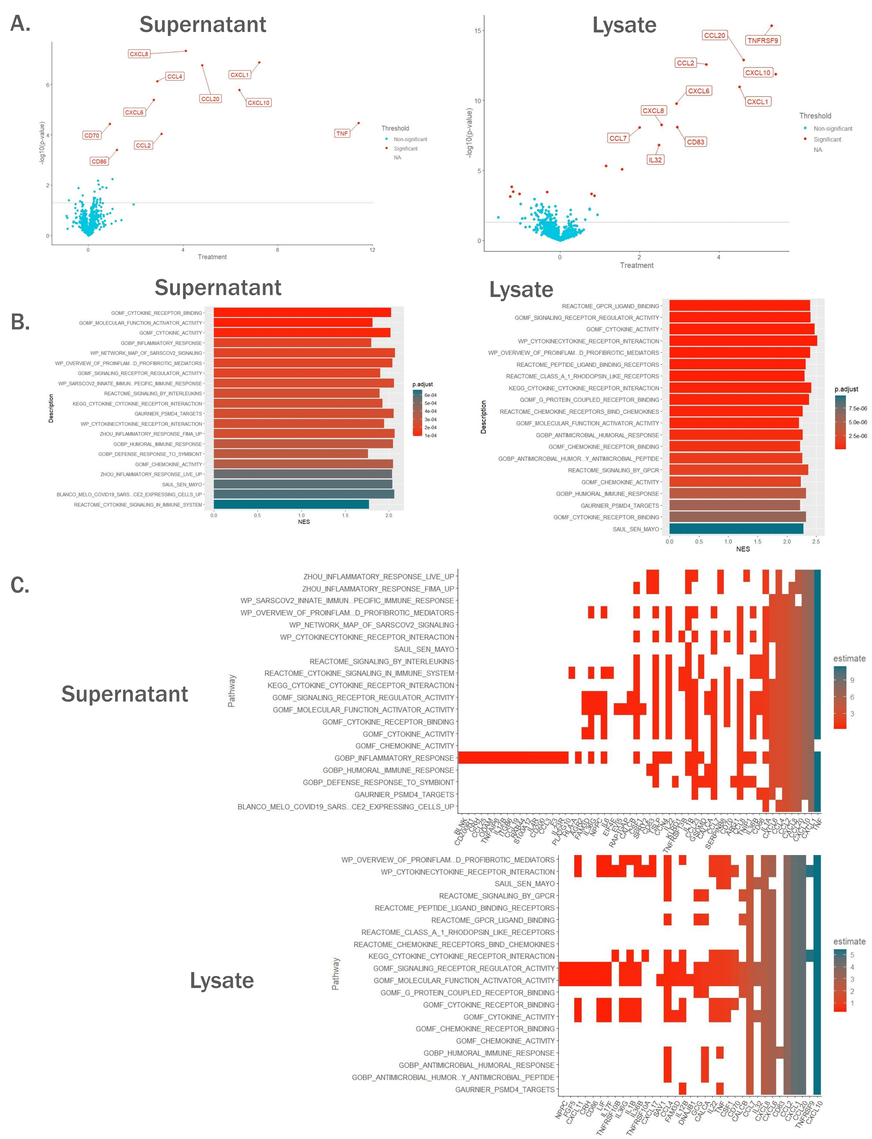

Figure 4. Cell culture supernatant and lysate proteomic analysis. Proteomic data from cell supernatant and normalized cell lysate generated with Olink Reveal was evaluated for differentially expressed protein (DEP) and pathway enrichment. (A) A volcano plot shows up-regulated or down-regulated proteins with the 10 most significant DEPs labeled. Elevated TNF-α in supernatant, but not in lysate aligns with the experimental dosage strategy. The other DEPs further support the evidence of enrichment of the NF-κB pathway highlighting chemokine-driven immune cell recruitment, amplification of inflammatory signaling, and cellular stress response. (B) Gene Set Enrichment Analysis (GSEA) reveals the top 20 enriched pathways, prominently featuring cytokine and chemokine activity alongside enhanced inflammatory and immune responses. (C) Heatmaps illustrate enriched pathways and their overlap with proteins showing significantly increased expression. Consistent with literature for this well-characterized signaling pathway, TNF-α treatment led to coordinated activation of cytokine signaling and enrichment of inflammatory response pathways across both the cellular and secreted proteome.

Conclusions

- We demonstrate a multiomic workflow to profile the cellular and secreted proteome in tandem with whole transcriptome profiling from a single sample.
- Observed changes in RNA and protein abundance following TNF-α treatment closely mirrors those expected in the canonical TNF-α signaling pathway.
- This study shows proof of principle for deploying high-plex proteomic and transcriptomic profiling to characterize inflammatory response in 2D and organoid cell cultures.