Differentiated transcriptomic signatures detectable in primary human hepatocytes transduced by AAV-based vectors: A potential enabler for *in vitro* safety profiling.

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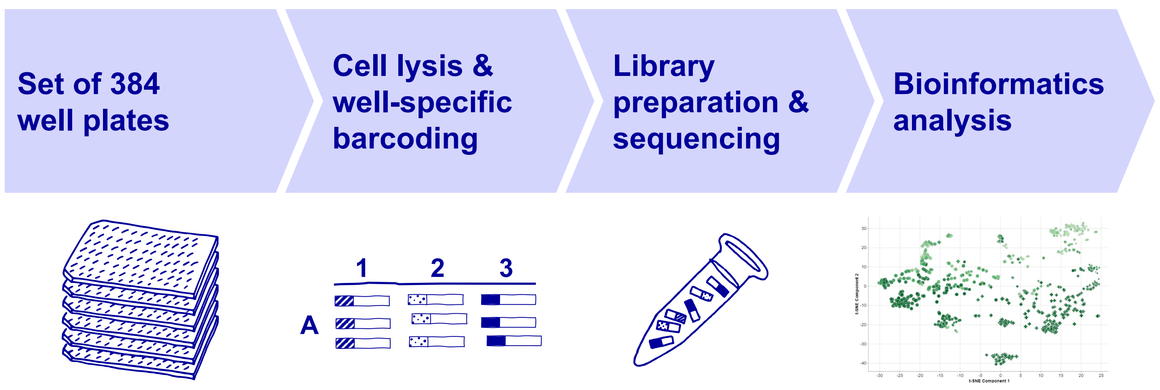
Visual Abstract



Introduction

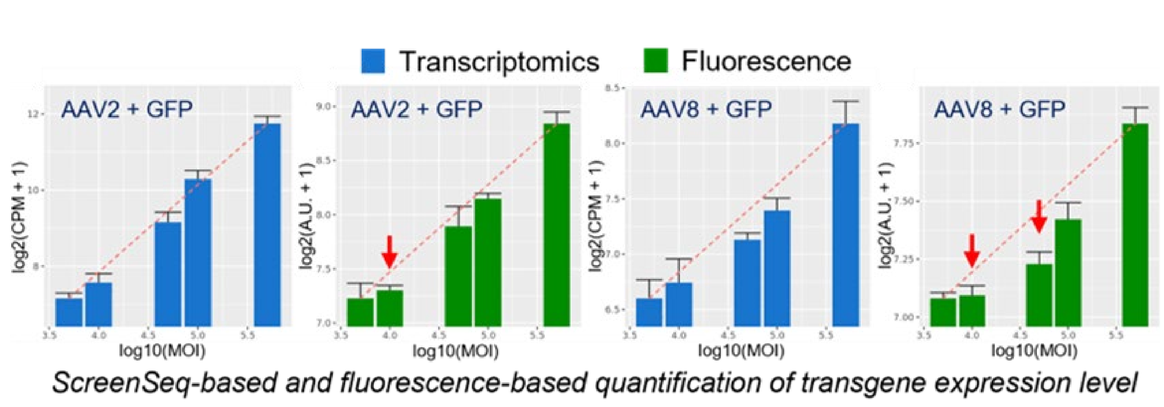
Multifactorial dose-related toxicities following treatments with AAV-based gene therapies have emerged in clinical trials and have raised concerns with regulators and health care providers. The reported adverse events are not limited to specific vectors, although liver toxicities are prominent and have led to discontinuation of clinical trials and serious events caused by already approved products[1]. However, the exact molecular pathologies leading to elevated liver enzymes, serious liver injury, and liver failure remain largely elusive. Clinical safety issues are poorly predicted by testing in preclinical tox species due to differences in biodistribution, transduction efficiencies, payload expression/clearance, and immunogenicity among others. Based on predictive Drug-Induced-Liver-Injury transcriptomics, we set out to build a defined in-vitro platform applicable to RNA sequencing of transduced human liver cells, with the aspiration to add molecular insights to vector safety assessments, aided and informed by transcriptomics profiling.[2,4]

Methods and Materials

Human liver cell lines (HepG2, HepaRG) and primary human hepatocytes (PHH) were seeded in 384-well plate formats and transduced by AAV2, AAV6, AAV8 and AAV9 based vectors expressing a gfp or luc reporter transgene (N= 8-16 per treatment). Control treatments included buffer controls and treatment with empty capsid (N=8-32/treatment). Time-course studies (up to 4 days) and vector titrations (MOI) were used to optimize transduction efficiencies and cell viabilities assessed by quantitative imaging and ATP measurements. All vectors transduced human liver cells, albeit with varying efficiencies (AAV6>AAV2>>AAV8>AAV9) and PHH were selected for transcriptomic profiling. RNA was isolated and subjected to unbiased high throughput RNA-Sequencing (htRNA-Seq). htRNA-Seq was done using ScreenSeq platform, quantifying expression levels for ca. 10,000 genes in a sample. Differential expression and Gene Ontology (GO) enrichment analysis (e.g., assessing stress response and effect on other intrinsic pathways) was done using a ML/AI supported bioinformatics platform (PanHunter).

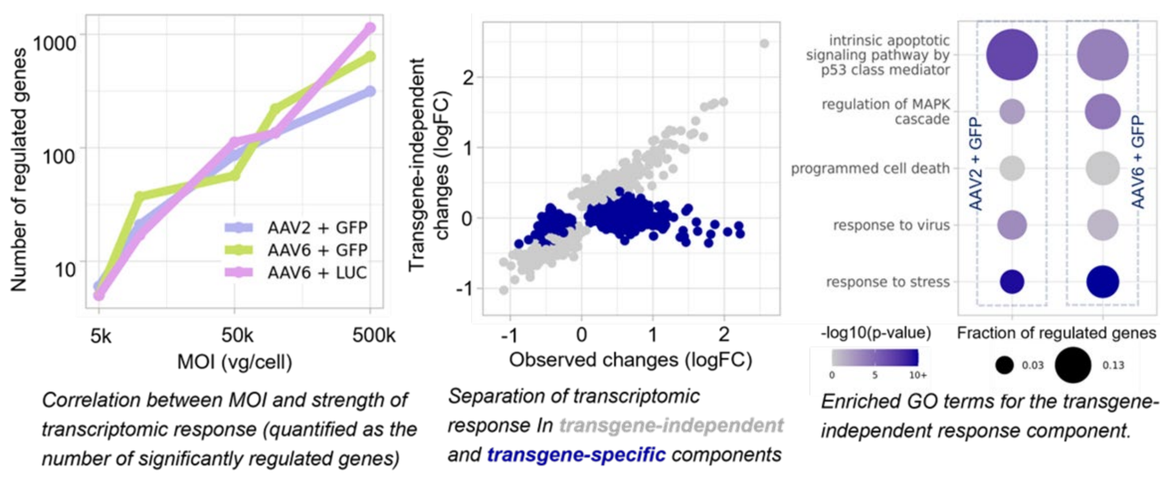
Results

ScreenSeq provides highly parallelized transcriptomic profiling for unbiased analysis of transcriptomic response to transduction with a correlation between multiplicity of infection (MOI) and strength of transcriptomic response.



ScreenSeq-based and fluorescence-based quantification of transgene expression level

Figure 1: htRNA-Seq enabled reliable phenotype-independent quantification of transgene expression, with sensitivity similar to or higher than sensitivity of e.g., fluorescence-based quantification.



Correlation between MOI and strength of transcriptomic response (quantified as the number of significantly regulated genes)

*Separation of transcriptomic response In transgene-independent and **transgene-specific** components*

Enriched GO terms for the transgene-independent response component.

Figure 2: A clear separation of response into transgene-independent and transgene-specific components was identifiable in PHH transduced by AAV-expressing reporter genes. Enriched GO terms for transgene-independent response components were identified in AAV-transduced PHH.

Conclusion

In summary this study in AAV-transduced PHH indicates that:

- Quantification of transcriptomic changes provides insight into differentiated responses to AAV vectors and their payloads
- Responses can be decomposed in transgene independent and transgene specific components
- Expanding this approach into liver micro-tissues and co-cultures of mixed cell types is feasible[3]
- Making use of machine-learning supported modelling[4], this approach could potentially be adapted to support the prediction of safety liabilities

References

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