Using single cell RNA-Seq data to identify cell type transcriptome specific changes in response to application of retinotoxins

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BACKGROUND

Retinal degenerative diseases (RDD) is one of the most common causes of blindness worldwide. Age-related macular degeneration (AMD) which is predicted to have affected 196 million people by 2020 is a type of RDD. While another form of RDD, Retinitis pigmentosa, affects 1 in 3000 people worldwide.

Current methods to investigate RDDs utilize **animal models,** such as zebrafish and mice**.** However, their retinas are **significantly different** from human retina. For example, they don't have a macula, and mouse retina only has two types of cones. Therefore, there is a need to discover new approaches in modeling RDDs.

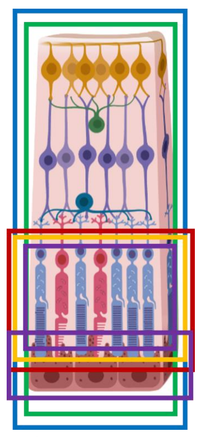


Figure 1. Retina layers. Source: BioRender

Human induced pluripotent stem cells (hiPSCs) are pluripotent stem cells derived from somatic cells, by introducing key transcription factors. HiPSCs have the **potential** to replace animal models in modeling RDDs. They can differentiate into retinal organoids, which mimic retinogenesis and contain all the key retinal cell types.

In this project retinal organoids are used as a potential model to reduce animal usage in toxicological studies. Retinotoxic drugs used in this research are **Digoxin**, **Sildenafil**, **Thioridazine**, **Methanol**, **and Ethanol.** Each of them affects the respective cell highlighted in their color. Generally, they manifest clinically as visual problems in intoxicated patients. The control drug used is **Ketorolac**, which has no effects on the retina.

AIMS

1. Use retinal organoids derived from hiPSCs as a human *in vitro* model for toxicological studies
2. **Utilize single cell RNA-Seq data to identify changes in the transcriptome of retinal cell types upon application of the six retinotoxins**

METHODS

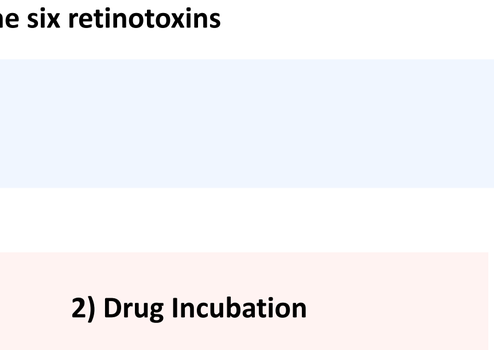
1) Retinal organoid differentiation

hiPSCs (Ad4 cell line) were differentiated into retinal organoids.

2) Drug Incubation

At day 200 of differentiation, retinal organoids were incubated with the retinotoxins for 24 hours, or with Sildenafil for 1 week. An additional control group was treated with PBS.

3) Single cell RNA sequencing at day 200



Differentially expressed gene lists were generated against control (PBS-treated).

4) Ingenuity Pathway Analysis (IPA)

- IPA Software analyzes the gene expression patterns using a build-in scientific **literature-based database.**
- **Core analysis** for retina cell types (Rods, Cones, Astrocytes, Retinal Ganglion Cells, Muller glia cells) was performed using processed scRNA-seq data.
- Analysis focuses on **retinal cell types.**
  - This poster focuses on **photoreceptors.**
- **Target genes, upstream regulators, and signaling pathways were identified.**

RESULTS & DISCUSSION

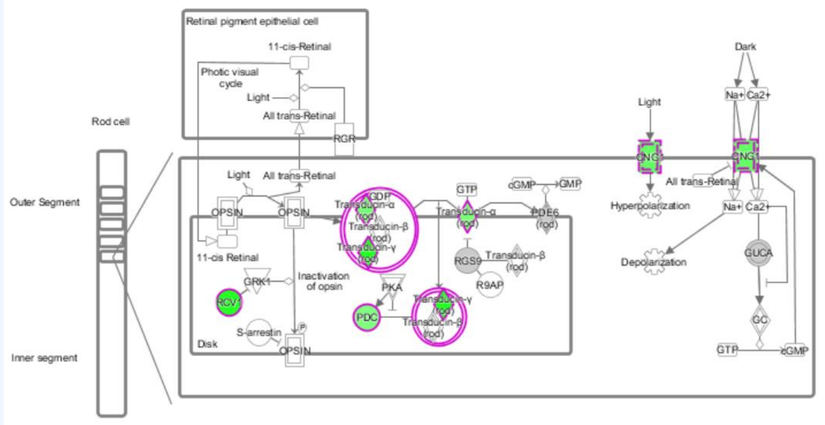
1. Retinotoxic compound effects on rod photoreceptors

Table 1. Summary of drug impact towards rod photoreceptors revealed by IPA.

	Target genes*	Upstream regulators	Signalling pathways
Digoxin	*IGFBP5,* ***NR2E3****, CRABP1, PTN, FABP7*	-	Retinoic acid mediated apoptosis signalling
Ethanol	*N/A*	N/A	N/A
Thioridazine	***CLU****,* ***GNGT1****,* ***NR2E3****,* ***RVCRN****,* ***RP1****,* ***CNGB1***	CRX	Phototransduction pathway
Sildenafil	***GNGT1****, RGS16,* ***CRYAB****, SOX2,* ***GFAP***	-	Phototransduction pathway
Methanol	*HES1, CLU, IGFBP2, PDE6A,* ***RP1***	CRX	Phototransduction pathway
Ketorolac	*IGFBP5, TTR,* ***NR2E3***	-	

*Red: Upregulated, Green: Downregulated

- **Cell stress** related genes were upregulated → *CLU, CRYAB, GFAP*
- **Key genes for retinal functions were** downregulated → *NR2E3, GNGT1, RP1, IGFBP5, RGS16, PDE6A, PTN*
- **Phototransduction process** was affected by **Thioridazine**, Sildenafil and Methanol → related with downregulation of key retina function genes (Figure 1)



Genes affected as seen in Figure 1:
- GNGT1 (↓)
- GNB3 (↓)
- GNAT1 (↓)
- CNGB1 (↓)
- PDC (↓)
- RCVRN (↓)

Figure 1. Example of phototransduction process affected by Thioridazine generated by IPA. Green indicates the downregulation of target genes which are key genes for normal retinal function.

2. Retinotoxic compound effects on cone photoreceptors

Table 2. Summary of drug impact towards cones photoreceptors revealed by IPA.

	Target genes*	Upstream regulators	Signalling pathways
Digoxin	***PCP2***	GNB3	-
Ethanol	*N/A*	N/A	N/A
Thioridazine	***RGR, TRPM1, AQP1***	NYX	Phototransduction pathway
Sildenafil	***CRYAB****, VIM, FABP7, SOX4*	-	-
Methanol	*TRH*	-	-
Ketorolac	*N/A*	N/A	N/A

*Red: Upregulated, Green: Downregulated

- Similar to rods, genes related with **key retinal functions** *(VIM, FABP7, SOX4, PCP2, TRH, RGR, TRPM1)* were downregulated and those related with **cell stress** *(CRYAB, GDF15)* were upregulated after incubation with retinotoxins.
- **Phototransduction pathway was** affected in cells incubated with **Thioridazine.**

CONCLUSION

- Retinotoxic compounds affects photoreceptors the most among the retinal cell types.
- Generally, genes related to stress were upregulated while genes related with key function of the retina were downregulated.
- Downregulation of key retinal genes led into dysfunction of Phototransduction process, which manifests as clinical symptoms commonly seen in toxicity.
- According to our data, retinal organoids can be utilized for toxicological studies.