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Oxidative Stress-Induced Dysregulation of Cytoskeletal Dynamics and Vesicular Trafficking in Retinal Pigment Epithelium: Implications for Retinal Degeneration

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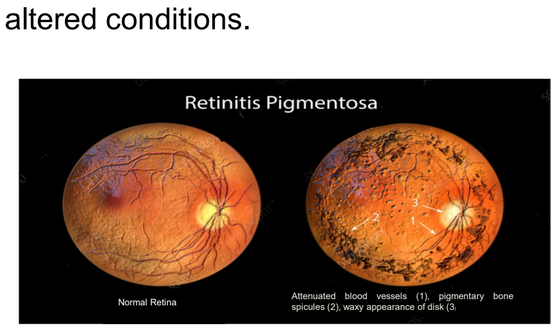
INTRODUCTION & AIM

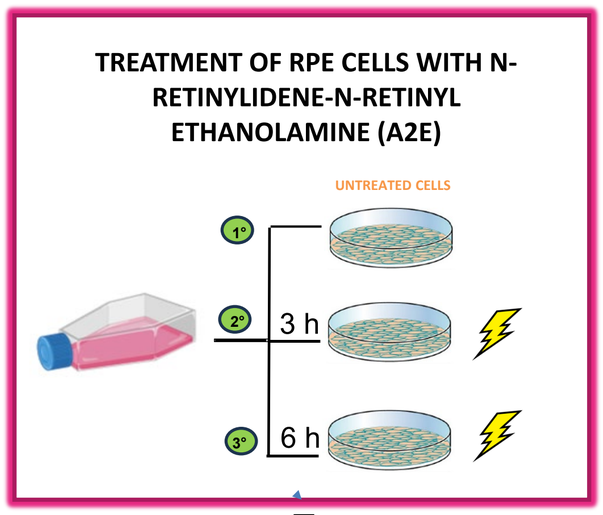
Retinal dystrophies like age-related macular degeneration and, particularly, retinitis pigmentosa represent a very heterogeneous group of ocular pathologies characterized by a very complex pattern of environmental and genetic causes. One of the most challenging aspects regards the incomplete knowledge of all causative genes and their involved biochemical and molecular pathways, leading to a huge group of orphan forms. Gene mutations or dysfunctional processes not only in the retina but also in RPE could cause inherited retinal degeneration, age-related macular degeneration and other retinal diseases. Such a feature highlights the relevant role of RPE, a high metabolic demand monolayer of pigmented cells that plays fundamental functions for both rods and cones, such as metabolite transport and photoreceptor excitability, regulation of visual cycle, secretion of growth factors, phagocytosis of photoreceptor outer segments (POSs) and oxidative stress defense. Regarding the latter point, oxidative stress represents one of the major lethal mechanisms responsible for age-related RPE damages. Current evidence suggests that RPE antioxidant defenses may fail to mitigate oxidative damage from oxLDL (oxidized low-density lipoprotein) and its metabolites, accelerating photoreceptor apoptosis. In this study, we treated RPE cells with oxLDL during a follow-up of two time points (3 h and 6 h) after exposure and compared them to untreated time zero controls. The main purpose of our experiment was the discovery of new pathways potentially involved in retinal dystrophies development, with the further detection of new candidate genes that could be associated or causative of such ocular diseases, emerging from the expression analysis in such altered conditions.

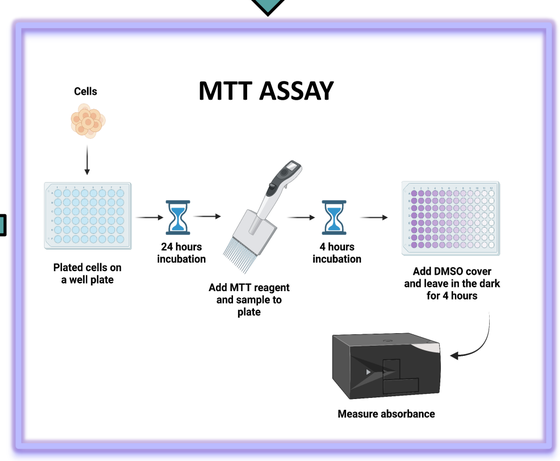


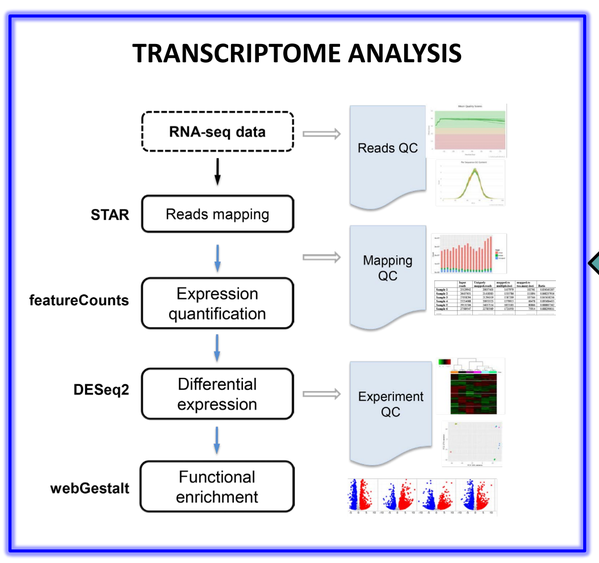
METHOD

Human RPE cells were exposed to oxLDL (100 µg/ml), and transcriptomic analyses were conducted across three time points (0h, 3h, and 6h). RNA-seq was utilized for differential gene expression profiling, and data were analyzed using Gene Set Enrichment Analysis (GSEA) to highlight significantly altered pathways.

- Establishment of cell cultures treated and untreated with oxLDL
- MTT Assay
- Total RNA Sequencing
- Quality Validation and Read Mapping
- Gene Expression and Statistical Analysis
- DE, DAS Analysis
- Gene-Enrichment and Functional Pathway Analysis
- Data Validation by qRT-PCR

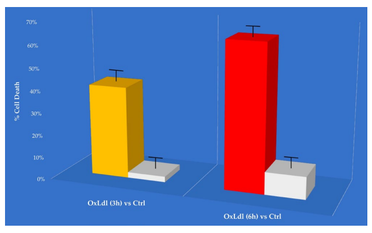




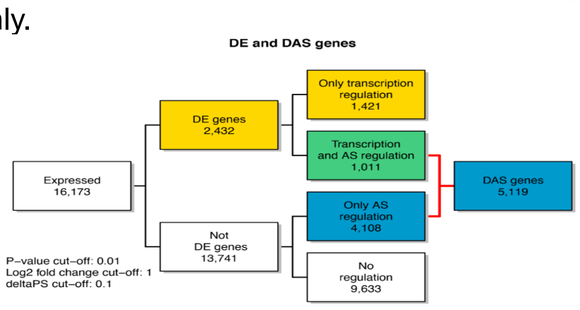


RESULTS & DISCUSSION

The MTT cell viability assay showed a relevant and different trend in RPE-treated cells versus control. The addition of oxLDL to cultures led to a dose-dependent increase in cell death percentage

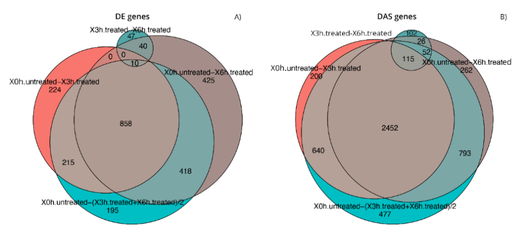


RNA sequencing carried out and a total of 16,173 genes and 69,653 transcripts were identified. We first analyzed differential expression at the gene level (DE) identifying 2432 genes that were significantly differentially expressed in response to oxLDL treatment. Of these, 59.7% resulted as up-regulated, while 40.3%
down-regulated. the analysis of transcript-level data allowed us to identify genes that were DAS between the contrast groups. We detected 5119 DAS genes, of which 1101 were also DE genes (regulated by both transcription and alternative splicing) and 4108 were regulated by alternative splicing only.

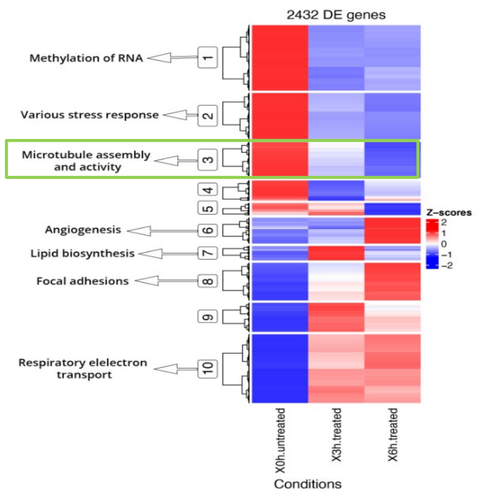


Consequently, it is evident how changes in gene-level expression and alternative splicing occurred throughout the whole period, either transiently (occurring after 3 h and returning to initial level after 6 h), occurring later (only after 6 h from treatment) or enduring throughout the whole period.

Such results probably suggest the different responses of RPE cells as a growing resistance to oxidative stress stimuli in a time-dependent manner.



Functional enrichment analyses of DE and DAS genes showed relevant differences. The most significantly enriched terms for DE genes were linked to cytoskeleton-associated genes and their potential roles in vesicular transport mechanisms and cellular signaling processes.
As evidenced by hierarchical clustering of total gene expression levels of DE genes, adaptive, transient and late expression profiles followed the oxLDL induced stress, and an analog response was highlighted by transcript expression profiles of individual DE genes.



The primary outcomes revealed:
1.Disruption of Retinitis Pigmentosa GTPase Regulator (RPGR), critical for ciliary protein transport, leading to impaired trafficking mechanisms;
2.Structural reorganization of actin filaments and destabilization of microtubule networks, which compromised cellular polarity and hindered vesicle mobility;
3.Elevated expression of microtubule-associated genes, implicated in photoreceptor disk formation and structural maintenance;
4.Gradual degradation of intermediate filament systems and adhesive junctions, resulting in dysfunctional communication between retinal pigment epithelium (RPE) and photoreceptors."

CONCLUSION

Pathways associated with cytoskeletal dynamics exhibit profound reorganization in response to oxidative stress, playing a central role in the disease mechanisms of Retinitis Pigmentosa (RP). Impairments in intracellular transport and cytoskeletal architecture highlight a key mechanism driving oxidative damage-induced retinal degeneration.

FUTURE WORK / REFERENCES

The findings could represent a significant step towards understanding the unclear molecular mechanisms linking oxidative stress and the etiopathogenesis of retinal dystrophies. This could potentially lead to the discovery of new therapeutic targets for these conditions.

Donato, L., D'Angelo R., Alibrandi, S., Rinaldi, C., Sidoti, A., Scimone, C. Effects of A2E-induced oxidative stress on retinal epithelial cells: new insights on retinitis pigmentosa development. Antioxidants 2020, 9, 307; doi:10.3390/ antiox9040307