

## Article

# Neuroretinal Cell Culture Model as a Tool for the Development of New Therapeutic Approaches for Oxidative Stress-Induced Ocular Diseases, with a Focus on Glaucoma

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**Abstract:** Glaucoma is a heterogeneous group of optic neuropathies characterized by a progressive degeneration of the retinal ganglion cells (RGCs), leading to irreversible vision loss. Nowadays, the traditional therapeutic approach to glaucoma consists of lowering the intraocular pressure (IOP), which does not address the neurodegenerative features of the disease. Besides animal models of glaucoma, there is a considerable need for *in vitro* experimental models to propose new therapeutic strategies for this ocular disease. In this study, we elucidated the pathological mechanisms leading to neuroretinal R28 cell death after exposure to glutamate and hydrogen peroxide ( $H_2O_2$ ) in order to develop new therapeutic approaches for oxidative stress-induced retinal diseases, including glaucoma. We were able to show that glutamate and  $H_2O_2$  can induce a decrease in R28 cell viability in a concentration-dependent manner. A cell viability of about 42% was found after exposure to 3 mM of glutamate and about 56% after exposure to 100  $\mu M$  of  $H_2O_2$  ( $n = 4$ ). Label-free quantitative mass spectrometry analysis revealed differential alterations of 193 and 311 proteins in R28 cells exposed to 3 mM of glutamate and 100  $\mu M$  of  $H_2O_2$ , respectively (FDR < 1%;  $p < 0.05$ ). Bioinformatics analysis indicated that the protein changes were associated with the dysregulation of signaling pathways, which was similar to those observed in glaucoma. Thus, the proteomic alteration induced by glutamate was associated with the inhibition of the PI3K/AKT signaling pathway. On the other hand,  $H_2O_2$ -induced toxicity in R28 cells was linked to the activation of apoptosis signaling and the inhibition of the mTOR and ERK/MAPK signaling pathways. Furthermore, the data show a similarity in the inhibition of the EIF2 and AMPK signaling pathways and the activation of the sumoylation and WNT/ $\beta$ -catenin signaling pathways in both groups. Our findings suggest that the exposure of R28 cells to glutamate and  $H_2O_2$  could induce glaucoma-like neurodegenerative features and potentially provide a suitable tool for the development of new therapeutic strategies for retinal diseases.

**Keywords:** glaucoma; neurodegeneration; oxidative stress; proteomics; retina



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## 1. Introduction

Retinal diseases are a significant health concern as they can cause irreversible blindness by affecting various parts of the retina [1]. These diseases are commonly caused by inflammation and oxidative stress or other pathological conditions that can lead to metabolic disorders [2,3], resulting in the death of retinal cells or adjacent supporting tissues. There are several types of retinal diseases, including age-related macular neurodegeneration (AMD), diabetic retinopathy (DR) and glaucoma.

Glaucoma is a group of eye diseases characterized by the progressive degeneration of retinal ganglion cells (RGCs) and an irreversible loss of vision [4]. Glaucoma affects more than 70 million people worldwide, with approximately 10% suffering from bilateral

# The New Culture Therapeutic Acti

**Robert R. Redfield**



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## **The New Culture Therapeutic Acti** Book Review: Unveiling the Power of Words

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