View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by IntechOpen

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

9

Glaucoma Genetics – Regulation of Cell Surviving and Death in the Retina

Maria D. Pinazo-Durán[1], Roberto Gallego-Pinazo[2], Vicente Zanón-Moreno[3,4], and Manuel Serrano[5]
[1]*Ophthalmic Research Unit "Santiago Grisolia", Valencia,*
[2]*Ophthalmology Department, University and Polytechnic Hospital La Fé, Valencia,*
[3]*Preventive Medicine and Public Health Department. Medical School, University of Valencia,*
[4]*CiberOBN - Biomedical Research Center Network of Pathophysiology of Obesity and Nutrition,*
[5]*Spanish National Cancer Research Center (CNIO), Madrid, Spain*

1. Introduction

Primary open-angle glaucoma (POAG) is a leading cause of various degrees of visual impairment and blindness worldwide, affecting in a disproportional manner women Afroamericans and Asians. In the Canadian Glaucoma Study, a recent multicenter prospective longitudinal study carried out in 258 participants (131 men versus 127 women; median age, 65.0 years), patients were followed up at 4-month intervals with perimetry, optic disc imaging, and a standardized interventional protocol for intraocular pressure (IOP) control. Univariate and proportional hazards models were used by the authors, in order to identify factors that predicted glaucoma progression. Data from this study showed that higher baseline age (HR per year, 1.04; 95% CI, 1.01-1.07), female sex (HR, 1.94; 95% CI, 1.09-3.46), and higher mean follow-up IOP (HR per 1 mm Hg, 1.19; 95% CI, 1.05-1.36) were associated with progression of glaucomatous disease (Chauhan et al., 2008).
It was estimated that over 8.4 million people were bilaterally blind from glaucoma in 2010, rising to 11.1 million by 2020. (Quigley & Broman, 2006). Moreover, approximately 50% of patients with POAG remain undiagnosed in most communities.
A wide spectrum of etiopathogenic theories have been proposed in relation to glaucoma. In the 19th century, Müller described that the elevated IOP caused a compression in the eye tissues, whereas chronic heightened IOP led subsequently to the neuronal death (*elder mechanical theory*), while simultaneously von Jaeger (1858) suggested that vascular alterations were responsible of the optic atrophy (*vascular theory*). Schnabel (1892) reported that it were created empty spaces during the process of atrophy of neural elements, which bowing back of the lamina cribrosa and posteriorly cupping the nervehead (*cavernous atrophy theory*). In 1925, La Grange and Beauvieux established that glaucomatous optic neuropathy was secondary to ischaemia (*ischaemic theory*). Changing criteria in the seventies

pointed to the role of altered axoplasmic flow in glaucomatous optic neuropathy. In fact, monkey eyes with a lesser elevation of IOP and shorter duration of glaucoma, showed changes sharply localized to the axon bundles in the scleral lamina cribrosa. Accumulation of mitochondria was detected anterior and posterior to collagenous septae. These changes co-localized to the sites of axoplasmic transport blockage, as identified by autoradiographic studies. It was speculated that these cytologic changes reflect interruption of axoplasmic flow in the optic nerve of glaucoma eyes, which raised the *new mechanical theory* (Gasterlaand et al., 1978).

The resistance of the trabecular meshwork (TM) to aqueous humour outflow increases as an ageing change, leading to increased IOP (Levin 1997). Therefore, elevated IOP is considered the main factor responsible for the glaucomatous optic neuropathy, this latter involving death of retinal ganglion cells and their axons. Clinically it is characterized by morphologic/morphometric changes of the optic disc, visual field defects (Agarwal et al., 2002) and increased rate of retinal nerve fiber layer thinning (Lee et al., 2011).

New extensive investigations into glaucoma pathophysiology contribute to our understanding of the role of a high variety of factors in the retinal ganglion cells damage and death. External and internal factors, as those within the retina and optic nerve ultrastructure, are important in the development and progression of primary open-angle glaucoma (POAG) (Lutjen-Drecoll et al., 1986; Hernandez et al., 1991; Triviño et al., 1996). More recent scientific knowledge revealed a complex situation in which other factors, as the circulatory (Yanagi et al., 2010), inflammatory (Kumarasamy et al., 2006), toxicologic (Schori et al., 2001), biochemical and molecular (Zanón-Moreno & Pinazo-Durán, 2008; Zanón-Moreno et al., 2008, 2009; Ray & Mookherjee, 2009, Osborne, 2010), are likely to be involved in the pathogenesis of glaucomatous optic neuropathy.

Whatever may be the real factors involved in glaucoma pathogenesis, the glaucomatous eyes suffer the dysfunction and death of the retinal ganglion cells leading to optic atrophy and irreversible visual loss. This may be the consequence of the association of multiple factors rather than only one functioning individually. In this context one question arises as to whether the molecular and cellular POAG basis, can be closely related to cell cycle abnormalities, leading to cell surviving and death involutionary processes, as a response to a cell stressor: the increased IOP.

1.1 The cell cycle

The cell-division cycle, are recognized as the events occurring in a cell that leads to its division and duplication (replication). The cell cycle consists of four phases: Gap 1 (G1) phase is the interval between mitosis and DNA synthesis, DNA synthesis (S) phase, Gap 2 (G2) phase (interphase) during which growth and preparation for cell division occurs, and finally the mitosis and cytokinesis (M) phase, as shown in the Fig. 1. During the cell cycle progression, activation of each phase is strictly dependent on the proper completion of the previous one. It has also to be stated that the cells that have temporarily stopped dividing have entered a stage of quiescence named the G0 phase.

Cell cycle is positively regulated by holoenzymes formed by a regulating subunit called cyclin (cyc), and cyclin-dependent kinases (cdk) (Ivanova et al., 2011). These complexes cyc/cdk become activated or inhibited sequentially in different phases of the cell cycle. Cell cycle progression is the result of the interaction between cyclins and their cdks, and a high variety of inhibitory proteins, the corresponding cdk inhibitors (cdki) (Lee et al., 2005). As

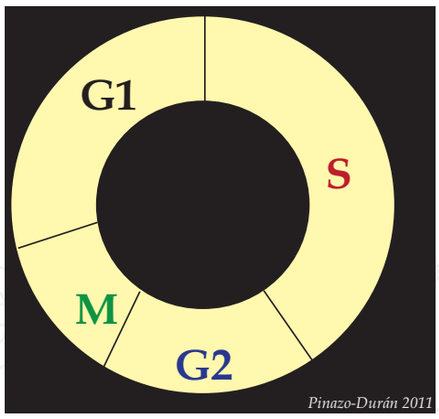


Fig. 1. The cell-division phases

shown in the following scheme, the cycD and the corresponding cdk4/6 activities regulate the early progression of the cell cycle, through the G1 phase. Then, cycE can be expressed, and in association with cdk2 controls the G1-S transition step. Finally, cell cycle arrest is achieved by the negative regulation of cdks, exerted either by antiproliferative signals or by the specific cdki activities (Serrano et al., 1993) (Fig. 2). Following growth arrest cells undergo senescence or apoptosis (Chen et al., 2000). The cip/kip family of cdki includes the genes p21CIP1/WAF (p21), p27 and p57, which halt cell cycle in G1 phase and inhibit several cyc/ckd complexes. The Inhibitor of Kinase 4/Alternative Reading Frame (INK4a/ARF) family prevent the progression of the cell cycle, and includes p16INK4a (p16), p15, p18, and p19, which specifically inhibit ccyc/dk4/6 activities (Serrano et al., 1993; Dean et al., 2010).

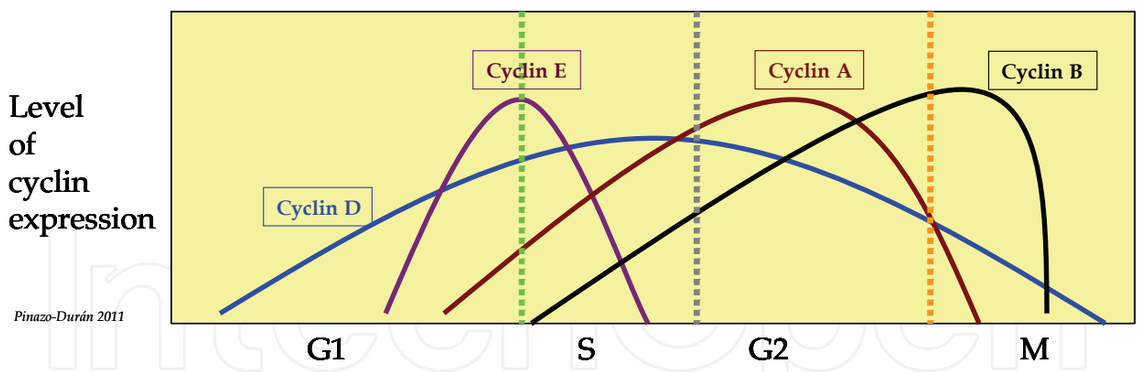


Fig. 2.

1.2 Genetics of glaucoma

Glaucoma is a multifactorial disease in which genetic and environmental factors are involved. Advancing knowledge in glaucoma is slow and insidious, because the polygenic character of the disease makes more difficult to progress through the genetic studies. However, genetics of glaucoma are the key for get preventing the glaucomatous blindness. It is necessary to deepen inside the influence of polymorphisms of each gene with the disease and, likewise, analyze the interactions among these genes (gene-gene) and through them with the environment (gene-environment).

Currently, there are some genes which have been associated to glaucoma, as myocilin/trabecular meshwork inducible glucocorticoid response **(MYOC/TIGR)** gene, that was identified in the latter nineties (Stone et al., 1997). The MYOC gene encodes an extracellular glycoprotein called **myocilin**. This protein is expressed in different human organs, among them the TM, ciliary body, retina and optic nervehead. Mutations in this gene result in juvenile glaucoma. The MYOC gene is altered in approximately 4% of cases of POAG (Lopez-Martinez et al., 2007).

Subsequently, other genes related to glaucoma were identified. Another causative gene was localized on chromosome 10p14 in a study of 54 families with autosomal dominantly inherited adult-onset POAG. It was designated **OPTN** (by its related protein, **optineurin**). The OPTN gene codes for a conserved 66-kilodalton protein of unknown function that has been implicated in the tumor necrosis factor-alpha signaling pathway and that interacts with diverse proteins including Huntingtin, Ras-associated protein RAB8, and transcription factor IIIA. Optineurin is expressed in the TM, nonpigmented ciliary epithelium, retina, and brain, and it was speculated that it plays a neuroprotective role (Rezaie et al., 2002). Moreover, sequence alterations in OPTN were found in 16.7% of families with hereditary POAG, including individuals with normal intraocular pressure

The WD repeat domain 36 (**WDR36)** gene that encodes a member of the **WDR protein family** was also found to be associated with glaucoma disease. WDR36 gene expressed in lens, iris, sclera, ciliary muscles, ciliary body, TM, retina and optic nerve, as established by real time-PCR. WDR36 is a novel causative gene for adult-onset POAG at the open-angle glaucoma (GLC1G) locus. Specific ocular expressions and observed mutations are consistent with WDR36 role in aetiology of both high- and low-pressure glaucoma (Monemi et al., 2005).

The **CYP1B1** gene encodes a dioxin inducible member of subfamily I of the **cytochrome p450** protein superfamily. The human CYP1B1 gene consists of three exons of which the first is non-coding. The putative open reading frame starts in the second exon and is 1629 bp in length, and the CYP1B1 gene has been related to 20-80% of primary congenital glaucoma cases (GLC3A) (Sitorus et al., 2003).

In addition to these four genes (MYOC, OPTN WDR36, CYP1B1) alterations in many others and their possible association with some type of glaucoma have been analyzed. There are studies involving the apolipoprotein E (ApoE) gene polymorphisms in the glaucomatous optic neuropathy. Copin et al., (2002) indicate that single nucleotide polymorphisms on the promoter region of the ApoE gene is associated with increased optic nerve and visual field damage in glaucomatous patients. This study also demonstrates that another single nucleotide polymorphism interacts with the recently reported MYOC single nucleotide polymorphism (mt1) that, in turns results in increased IOP, suggesting that may be involved in the limited effectiveness of IOP-lowering treatments among POAG patients. More recently it has been shown that heterozygous non-synonymous variants of the retinitis pigmentosa GTPase regulator interacting protein 1 (RPGRIP1) gene also may cause, or increase, the susceptibility to various forms of glaucoma (Fernández-Martínez, 2011).

At the same time, we shouldn't ignore epigenetics, which regards to the study of those heritable changes in phenotype or gene expression caused by processes different to variations in the DNA sequence. Epigenetic information modulates gene expression without alter the DNA sequence, by several mechanisms: 1) DNA methylation, 2) Genomic imprinting and 3) Histone modifications –acetylation, methylation, phosphorylation-. Therefore, epigenetics does refer to the mechanisms that utilize the organism for translating the genetic

information of a generation. The clinical effects that arise from the disease-associated sequence changes in gene might be influenced by modifier genes, as well as by endogenous factors that could alter pathogenic mechanisms affected by the mutations. Therefore, it could potentially explain some variations in the observed clinical signs and symptoms and also in the progression rates of the POAG patients (Lam et al. 2000).

Moreover, from a nutrition and health viewpoint, preventive strategies from population-based recommendations to personalized nutritional advice are detected as an attractive topic. Biomedical studies may help in understanding how genetic variation and epigenetic events may change requirements and responses to nutrients, and developing outstanding techniques for identifying patients at risk because of its own nutrigenetic profile (Zeisel, 2007). A discipline that is gaining importance in glaucoma studies is the nutritional genetics, which has two main branches: nutrigenetics and nutrigenomics. Nutrigenetics focuses on the study of genetic variations in the body's response to nutrients. Otherwise, nutrigenomics investigates the influence of nutrients on gene expression. In relation to these two concepts especial attention has to be paid to the emergence of nutrigenetic tests, which use genetic information to identify food products that suited or not suited to the personalized nutrigenetic profile, allowing us to define individual dietary advice (Haga et al, 2003).

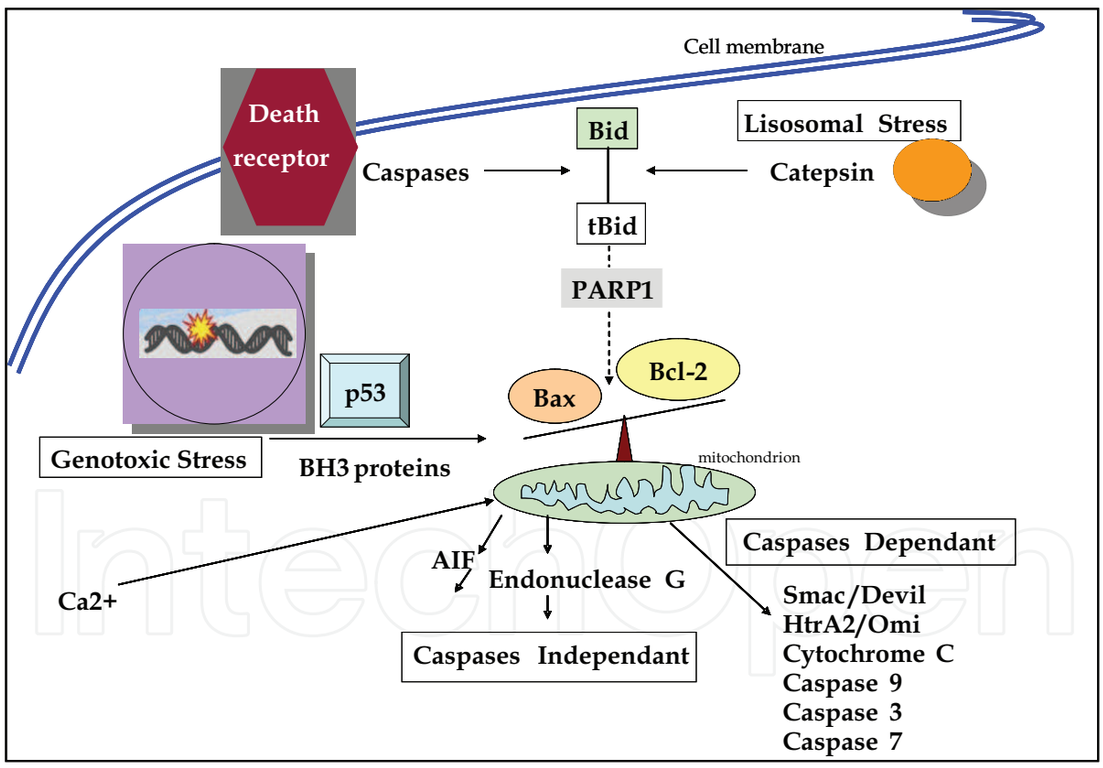


Fig. 3.

Numerous studies on gene alterations that encode proteins related to cell survival and death in relation to glaucoma have been conducted through the past twenty years, in order to stop or prevent apoptosis of retinal ganglion cells and optic nerve degeneration. In this regard, it has been analyzed mutations in genes of the B-cell lymphoma 2 (bcl-2) family such as the B-cell lymphoma 2-associated X (BAX) gene, which encodes a pro-apoptotic X protein. The

circuits involved in these cell pathways are shown below (Fig. 3). If the BAX protein is not present, or there are BAX limited availability, ganglion cell somas are capable to resist indefinitely an optic nerve insult. In the presence of BAX the damage cannot be blocked and cell death program reaches an irreversible point (Nickells, 2008).

1.3 The p53 gene

Another key protein in the regulation processes of cell survival and death is p53. It was identified in 1979 and encodes the transcription factor also named p53, a phosphoprotein (MW 53 kD). The p53 gene comprises 20 kb of DNA and is located on the short arm of chromosome 17p13.1 (Levine and Finlay 2004). It has been called the "guardian of the genome" (Lane 1992). Its expression and function has been related to apoptosis, cell proliferation and/or angiogenic processes.

The p53 protein is located in the cytoplasm as an inactive monomer, but upon stimulation by a variety of cellular stresses, p53 forms a tetramer from double dimers. The p53 protein binds DNA (see the figure 4) and, among other target genes, induces the expression of the p21 gene to synthetize the p21 protein that, in turns, inhibits the cycE/cdk2 complexes. The complex p21/cycE/cdk2 inactivates cell division by stopping the cell cycle. p53 is an essential molecule in cell proliferation and apoptosis (Guevara et al., 1999). Inactive, as a good guardian, p53 only starts functioning when a genetic damage appears in a cell (Fig. 3,4). Only then, it activates a system with two main programs: 1) The cell suicide, 2) A process of cellular senescence by which the cell remains alive but unable to proliferate. The p53 gene detects almost any alteration in the cell, oncogenes and other DNA alterations, lack of nutrients, and abnormalities that occur during the proliferative and angiogenic processes. Therefore, p53 is a multifunctional “star” gene, involved in a wide spectrum of biomedical facts (Boesten et al., 2009; Gallego-Pinazo et al., 2008, 2010; Jeong et al., 2009). The tumour suppressor p53 mediates the cellular response to a variety of stresses mainly by regulating

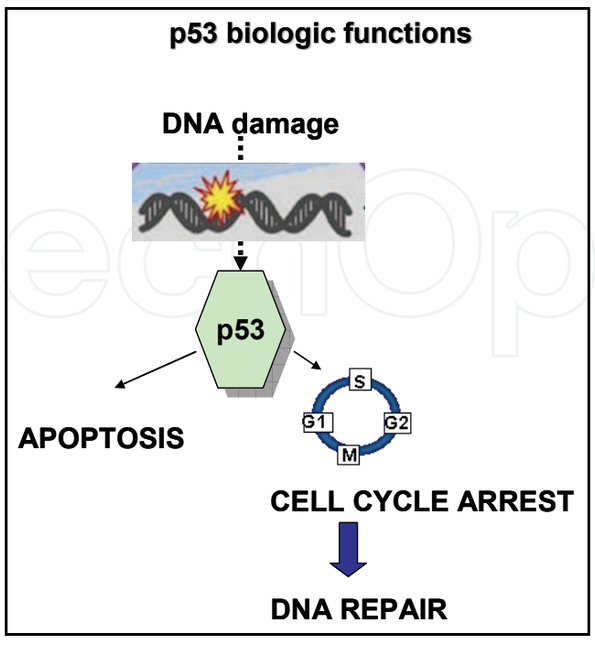


Fig. 4.

the transcription of over 150 genes (Jeong et al, 2009). Active p53 gene can induce reversible growth arrest in the cell cycle G1 or G2 phases, as well as cellular senescence or apoptosis. As such, p53 acts as a sensor of damage to the genome (Lane 1992). Main biologic functions of p53 are shown in the scheme below.

1.4 Glaucoma, visual loss and apoptosis

In a first clinic-pathologic report Levin and Louhab (1996) identified retinal ganglion cells undergoing apoptosis in one of the eyes of a 70-year-old man with anterior ischaemic optic neuropathy. These authors speculated that the affected eye underwent a functional optic nerve axotomy. A wide variety of clinical and experimental studies strongly indicate that retinal ganglion cells and optic axons demise represents the final pathway of vision loss in glaucoma patients (Quigley et al., 2000; Agar et al., 2006; Ju et al., 2007; Agarwal et al., 2009, Lee et al., 2011) and that the intrinsic pathogenic mechanism is the ganglion cell death by apoptosis (Nickells 1999; Osborne et al., 1999; Osborne, 2011; Zhang & Bhavnani 2005; Levkovitch-Verbin 2009; Tatton, 2011). Although there are compelling evidences showing apoptosis as the primary and early mechanism of ganglion cell death in glaucoma (Levkovitch-Verbin 2006), necrosis is also a contributory mechanism in the late phase of glaucoma progression, evidence to which was observed in rats subjected to optic nerve transection (Cordeiro et al., 2010).

When managing glaucoma patients, ophthalmologists consider individualized therapy for lowering IOP, as well as the true IOP-lowering efficacy of each drug that is given to any patient, and the compliance degree, with the final goal of maintaining the adequate IOP levels and subsequently the vision and the quality of life (Levin 1997; Agarwal et al., 2009). However, the anti-hypertensive therapy is clearly unable to properly maintain the visual function. Further research and translation of knowledge to the clinical practice in urgently needed to improve glaucoma prognosis.

Apoptosis (Kerr et al., 1972) is a regulated manner to destroy a cell. During development apoptosis is a pivotal process. In addition, apoptosis performs essential functions in morphogenesis and tissue remodelling, homeostasis, removal of damaged or infected cells, and others. Although it is essential for normal development and health, an aberrant activation contributes to the pathogenesis of ischemic or neurodegenerative diseases and other processes (Walker et al., 1988). On the contrary, the apoptosis failure is a key factor in the pathogenesis of cancer or autoimmune disorders (Wyllie 1974). Apoptosis, is caused by the production of an endonuclease that destroys the DNA and induces cell phagocytosis by the neighboring elements, but without stimulating inflammation (Birge & Ucker 2008). In this context, visual impairment and blindness in glaucoma patients is attributed to the retinal ganglion cell damage and death (McKinnon, 1997).

Apoptosis is a genetically coded cell suicide program that activates when any cell has been profoundly damaged or the cell is no longer needed. Microscopic findings of apoptotic cells include: 1) cytoplasmic organelles compaction, 2) nuclear chromatin condensation, and 3) membrane blebbing (Meagher et al., 1992).

Furthermore, neurotrophin withdrawal and cytotoxic neurotransmitters release have also been involved in ganglion cell and optic fibers damage and death by apoptosis, in glaucoma (Carmignoto et al., 1997). Among the neurotrophic factors the BDNF is a pivotal molecule for neuron survival, in the developing and mature visual system (Snider 1994; Carmignoto et al., 1997). The BDNF was investigated in rats and monkeys regarding the retinal ganglion cells neurotrophin transport in experimental glaucoma, by using acute and chronic IOP

elevation and immunohistochemical assays with antibodies directed against the tyrosine kinase receptors (TrkA, B, C) and against BDNF, as well as by autoradiography to identify retrograde axonal transport of 125I-BDNF injected into the superior colliculus. The authors concluded that the interruption of BDNF retrograde transport and the accumulation of TrkB at the optic nervehead in acute/chronic glaucoma models strongly suggest a role for neurotrophin deprivation in the pathogenesis of glaucomatous ganglion cell death (Pease et al., 2000).

Recently it has been stressed the importance of identifying molecular biomarkers or inducers of apoptosis for better glaucoma managing (Golubnitschaja & Flammer, 2007; Zanón-Moreno & Pinazo-Durán, 2008). Therefore, different classes of molecular anomalies can be detected and utilized for glaucoma progression, among them specific molecules closely related to cell survival and apoptosis, as follows.

Caspase-3 is one of the cysteine proteases family that plays a role in apoptosis by cleaving a high variety of key proteins such as the poly (ADP-ribose) polymerase 1 (PARP-1), protein kinase C (PKC), DNA-dependent protein kinase, DNA-fragmentation factor (DFF) and others (see the drawn schematic on figure 3). Zang and Bhavnani (2005) described that up-regulation of caspase-3 expression preceded neuronal cell death, supporting the possibility that glutamate-induced apoptotic cell death was the consequence of up-regulation of caspase-3 gene in cortical neurons. These observations are consistent with up-regulation of precursor caspase-3 in frontal neuronal cortex of subjects with Alzheimer's disease. This enzyme has been proposed to activate death effector molecules resulting in the fragmentation of genomic DNA and was associated with morphological and structural changes characteristic of apoptosis.

Poly-Adenil Ribose Polymerase 1 (PARP1) is a reversible post-translational protein modification that is involved in the regulation of several biological functions. Whereas an 18 member superfamily of PARP enzymes synthesize poly (ADP-ribose) (PAR), a single protein, PAR glycohydrolase (PARG) is responsible for the catabolism of the polymer. PARP-1 accounts for more than 90% of the poly (ADP-ribosyl)ating capacity of the cells (Viraq 2005). PARP-1 activated by DNA breaks cleaves NAD(+) into nicotinamide and ADP-ribose, and uses it to synthesize long branching PAR polymers covalently attached to acceptor proteins including histones, DNA repair enzymes, transcription factors and PARP-1 (Aguilar-Quesada et al., 2007). Activation of PARP-1 by mild genotoxic stimuli may facilitate DNA repair and cell survival. However irreparable DNA damage triggers apoptotic or necrotic cell death (as reflected in figure 3). In apoptosis, early PARP activation may assist the apoptotic cascade, by stabilizing p53, by mediating the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus or by inhibiting early activation of DNases. In most severe oxidative stress situations, excessive DNA damage causes over activation of PARP-1, which blocks the apoptotic elements and switches the mode of cell death from apoptosis to necrosis. In addition to serving as a cytotoxic mediator, PARP-1 is also implicated in transcriptional regulation processes, most notably in the NF kappaB and AP-1 driven expression of inflammatory mediators (Viraq 2005). In the case of pharmacological blockage or genetic inhibition of PARP-1 provided remarkable protection from tissue injury in various oxidative stress-related disease models ranging from endothelial dysfunction, myocardial ischaemia-reperfusion, stroke, shock, diabetes mellitus, Parkinson's disease, arthritis, and uveitis. These beneficial effects are attributed to inhibition of the PARP-1 mediated suicidal pathway and to reduced expression of inflammatory cytokines and other molecules (Quiles-Perez et al., 2010).

Despite former glaucoma statements, it has been suggested that within the retina, other cell phenotypes that the ganglion cells may be implicated. There is also increasing evidence that neuronal changes occur both in retina and central visual pathways in glaucoma and other neurodegenerative diseases. Axonal projections can be distinguished from dendrites by the shape, length and function. Dendrites are fine neuronal processes which support postsynaptic contact elements and are responsible for receiving synaptic signals. The morphological and functional integrity of dendrites has important effects on integrating neuronal input to the CNS from the peripheral targets. Axonal and dendritic changes have been detected in neurodegenerative processes, including those occurring in development, ageing and diseases. Therefore, axonal and dendritic pathology are early signs in disease and providing new insights into therapeutic strategies. Increased latency and reduced amplitude of visual evoked potentials, frequently encountered in ocular hypertension or POAG, suggest slowed neural conduction in the visual pathways (Parisi 1997; Bach and Hoffmann 2008). Recent research in monkeys demonstrated that the glaucomatous damage extends from the retina to the visual centers in the brain, but being the primary region of damage the optic nervehead, with the lateral geniculate nucleus being secondarily affected. These findings indicate that in Japanese monkeys, damage to neurons in the geniculate can be detected in a very early phase (first weeks) after an IOP elevation occurs, as can damage to optic nervehead (Itoa et al., 2009). By utilizing neuroimaging techniques such as diffusion tensor magnetic resonance imaging, functional magnetic resonance imaging, and magnetic resonance spectroscopy it has been evaluated the microstructural integrity of white-matter fibers and the functional activity of gray matter. They have been widely employed to investigate various diseases of the central nervous system, as glaucoma. It has been demonstrated alterations involving the human visual cortex that are consistent with clinically documented losses of visual function. These data support the use of imaging techniques as reliable, noninvasive tools for monitoring the progression of human glaucoma (Garacci et al., 2008)

In this context, there may be new opportunities to develop treatments directed at the retina and the brain, such as those that promote healthy ocular tissues, the neurotrophic factors.

Neurotrophic factors includes: 1) neurotrophins (NGF, BDNF, NT-3, NT-4), 2) neurokines (CNTF), and glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) (Bespalov and Saarma, 2007). Neurotrophic factors and their receptors regulate the development and physiology of neurons, but their roles in the CNS are far from complete. Among the neurotrophins, the brain derived neurotrophic factor (BDNF) (Snider 1994; Osborne et al., 1999; Harada et al., 2011) may help retinal ganglion cells to survive themselves; may increase axon outgrowth in the optic nerve, and may improve the environment in which the dying retinal ganglion cells and the surrounding elements are placed within the retina, optic nerve and visual pathway.

Considering that the cell surviving and death mechanisms play a pivotal role in glaucoma progression, we propose that the p53 gene, as the outstanding cell cycle regulator, can be involved in the pathogenic processes of glaucoma in the retina and optic nerve. Therefore, we deal with examining the events that take place in the retina at the molecular level, after overexpressing p53 gene, with the inhibition of the cell cycle progression and the induction of the apoptosis-related pathway.

2. The super p53 mice

The C57BL6 strain is the most commonly used for genetic manipulation and biomedical research. Transgenic mice of the Charles River C57BL6/J strain were generated by addition

of two extra copies of p53 gene (García-Cao et al., 2002; Effeyan et al., 2006). Our main purpose is to characterize molecules involved in cell surviving and death in mice retina-choroid.

2.1 Animal and tissue handling

All procedures were performed to minimize animal suffering in accordance with the European Community guidelines for the use of animals in research (609/1986). The Research Committee and Animal Research Committee of the participating centers approved the study. Mice were sacrificed and utilized by several investigators in our working area. For the purposes of the present study we utilized 24 mice that were distributed into two groups 1) super p53 (sp53; n=12) and wild type (CG; n=12).

Eyes with the retrobulbar optic nerves were microsurgically dissected, in a Nikon SM2 1500 microscope. Briefly, after performing an "ab externo" enucleation, the eyes were washed in bidistilled water and placed on a Petri dish to be immobilized by means of a Barraquer forceps to perform an anterior segment paracentesis with a micro sharp blade angled 15º. By using the Vannas scissors a complete peritomy was performed, to separate the cornea and lens from the posterior eyecup (with the optic nerve attached), as previously described (Pinazo-Durán et al., 1993, 1997, 2005, 2011). The retina-choroid was obtained, and frozen and stored at -80º until processing. Homogenates of the retina-choroid were used to perform two main techniques:

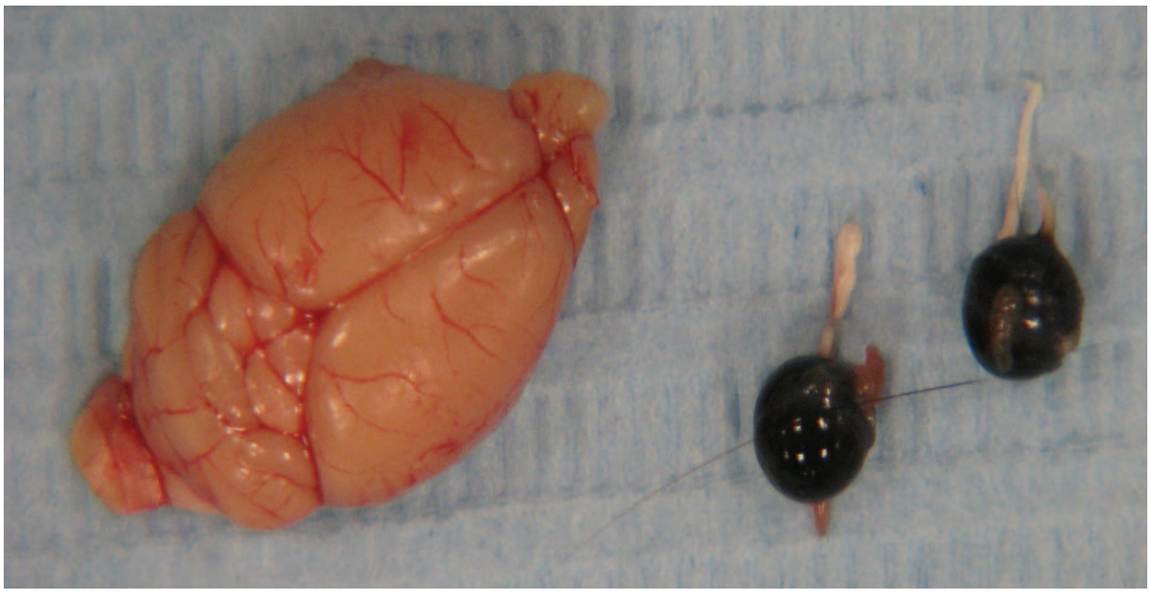


Fig. 5. The super p53 mice brain, eyeballs and optic nerves

1) Enzyme-Linked ImmunoSorbent Assay (ELISA) for determining the BDNF levels in the ocular tissues, and 2) Western blot and immunoblotting procedures by determining the total protein concentration in the eye tissues, and SDS Page with transferring to nitrocellulose membranes, incubating alternatively with anti PARP1 and anti CS3 antibodies, as previously described by Laemmli (1970) and with some respectful modifications for the eye tissue processing by Pinazo-Durán et al., (1996). The expression bands of both molecules were alternatively examined and quantified by laser densitometry.

2.2 Expression of the BDNF, PARP1 and CS3 in the sp53 retina-choroid

The ELISA assay allowed us to detect the BDNF concentration in the retina-choroid of both groups of mice. It was observed that the BDNF expression was significantly higher in the sp53 tissues than in the corresponding controls, as reflected in the Table 1.

Molecules	Sp53G	WTG	p-value
BDNF (pg/mL)	121,13 ± 2,72	108,16 ± 4,43	0,00250
PARP-1 (laser rdu)	143,28 ± 5,38	105,46 ± 3,91	0,00003
CS-3 (laser rdu)	169,20 ± 3,17	167,22 ± 1,40	0,29600

Table 1.

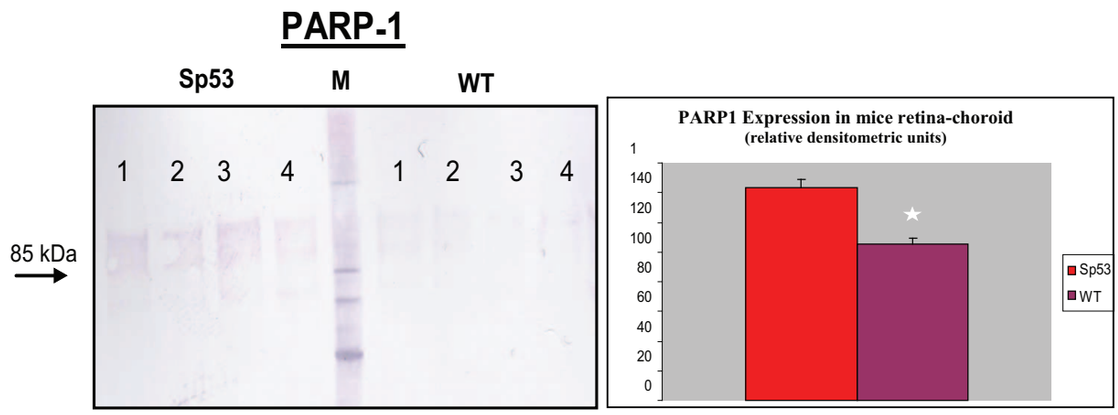


Fig. 6. Immunobloting in mice retina choroid for PARP1 expression and the corresponding quantitative results by laser spectrometry

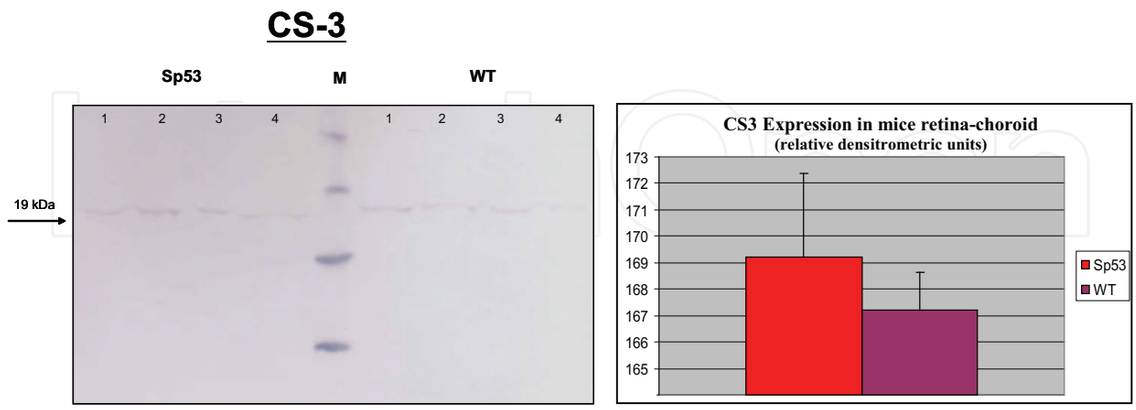


Fig. 7.

The results showed that the two enzymes involved in the apoptotic cascade, PARP1 and CS 3 expressed differently in the retina-choroid of the two groups of mice, depending on the genetic background. When the nitrocellulose membranes were incubated, revealed and examined, the corresponding bands of the PARP1 and CS3 expression were more noticeable

in the sp53G than in the wtG eyes, data confirmed by the quantification analysis performed by laser densitometry (relative densitometric units), as can also be observed in the above table 1. CS3, although there was more noticeable in mouse tissue samples sp53, showed no significant differences with the wild mouse having normal p53 genetic load (figures 6 and 7).

3. Cell surviving and death

As mentioned previously, several mechanisms have been implicated in initiating the apoptotic cascade in glaucoma. These mechanisms and their potential therapeutic effects include inflammation, ischaemia, excitotoxicity, oxidative stress, mitochondrial dysfunction, and neurotrophin deprivation. Taking into consideration that apoptosis significantly contributes to ganglion cell loss in glaucoma, and in the case that the specific apoptosis signalling pathways for glaucoma can be known (Ray & Mookherjee 2009), any agent that can help to block or interrupt the specific signalling, may have the potential to slowing or stopping glaucoma progression and visual loss.

Charles et al., (2005) investigated the apoptosis-related signaling pathways in a cultured rat retinal ganglion cell (RGC-5) line deprived of growth factors after serum withdrawal from the culture medium. The authors described that serum withdrawal induces apoptotic cell death in RGC-5 cells, via mitochondrial pathways, leading to the speculation that growth factor deprivation arising from blockade of retrograde transport of neurotrophins, may involve similar mechanisms of retinal ganglion cell death in glaucoma. Khalyfa et al., (2007) induced apoptosis in the retinal ganglion cells in transformed rats in order to generate a genome-wide gene expression in cultures of retinal ganglion cells, following serum deprivation and to identify candidate genes that may be involved in the signal transduction pathways.

Thus, the genes identified in microarray data and validated by real-time RT-PCR may play an important role in retinal ganglion cell death. Among the validated genes, C3 and C1s showed significant upregulation of the complement component pathway. The results further indicate that components of the complement pathway are present in neurons of the rat retina.

It would be appropriate also in the context to cite that Erythropoietin modulates erythropoiesis by inhibiting apoptosis in erythrocyte progenitors. This molecule has been demonstrated to be protective in experimental models of trauma, cerebral and retinal ischaemia and neuroinflammation. Moreover, it was described that erythropoietin promoted retinal ganglion cell survival, without affecting IOP, in DBA/2J glaucomatous mice, suggesting that erythropoietin may be a potential therapeutic neuroprotectant in glaucoma (Zhong et al. 2007).

It may be speculated that determining the regulation of cell survival and death mechanisms in the retina it may be throw some light on the pathogenesis of POAG. In this context, the elevated IOP leads to molecular events that are destructive to the retinal ganglion cells and optic axons, but also induces specific changes that are potentially protective against cellular injury and death. If this is the case, then the glaucomatous retinal ganglion cell death and subsequent axonal loss would result not only from initiation of apoptosis, but also from failure of intrinsic cell protective mechanisms.

Consequently, it should therefore be theoretically probable to act on the regulation processes of these two classes of responses, and improving ganglion cells survival when glaucoma develops, as well as in glaucoma progression. Since the transgenic mouse used in the present work, which has 4 copies of the p53 gene, displayed a significantly higher

expression of molecules involved in cell surviving and death in the retina, we suggest that p53 may be involved in regulation of these processes. Our findings may open new diagnostic and therapeutic possibilities by the p53 related biomedical and biotechnological applications in glaucoma.

4. References

Agar A, Li S, Agarwal N, Coroneo MT, Hill MA, Retinal ganglion cell line apoptosis induced by hydrostatic pressure, *Brain Res*. 2006; 1086:191-200.

Agarwal R, Gupta SK, Agarwal P, Saxena R, Agrawal SS. Current concepts in the pathophysiology of glaucoma. *Indian J Ophthalmol* 2009 57:257-266

Aguilar-Quesada R, Muñoz-Gámez JA, Martín-Oliva D, Peralta-Leal A, Quiles-Pérez R, Rodríguez-Vargas JM, Ruiz de Almodóvar M, Conde C, Ruiz-Extremera A, Oliver FJ Modulation of transcription by PARP-1: consequences in carcinogenesis and inflammation. *Curr Med Chem.* 2007; 14:1179-87.

Bach M, Hoffmann MB. Update on the pattern electroretinogram in glaucoma. *Optom Vis Sci*. 2008; 85:386-395.

Baltmr A, Duggan J, Nizari S, Salt TE, Cordeiro MF. Neuroprotection in glaucoma -Is there a future role? *Exp Eye Res.* 2010 ; 91:554-566.

Bespalov M & Saarma M. (2007). GDNF receptor complex is an emerging drug target. *Trends in Pharmacol Sci* 28: 68-74.

Birge RB, Ucker DS. Innate apoptotic immunity: the calming touch of death. *Cell Death Differ* 2008 15, 1096–1102

Boesten LS, Zadelaar AS, van Nieuwkoop A, Hu L, Teunisse AF, Jochemsen AG, Evers B, van de Water B, Gijbels MJ, van Vlijmen BJ, Havekes LM, de Winther MP. Macrophage p53 controls macrophage death in atherosclerotic lesions of apolipoprotein E deficient mice. *Atherosclerosis*. 2009; 207:399-404.

Carmignoto G, Pizzorruso T, Tia S, Vicini S. Brain derived neurotrophic factor and nerve growth factor potentiate excitatory synaptic transmission in the rat visual cortex. *J Physiol* 1997; 498 153-164.

Charles I, Khalyfa A, Kumar DM, Krishnamoorthy RR, Roque RS, Cooper N, Agarwal N. (2005) Serum deprivation induces apoptotic cell death of transformed rat retinal ganglion cells via mitochondrial signaling pathways. *Invest Ophthalmol Vis Sci* ; 46 :1330-1338.

Chauhan BC, Mikelberg FS, Balaszi AG, LeBlanc RP, Lesk MR, Trope GE; Canadian Glaucoma Study Group. (2008). Canadian Glaucoma Study: 2. risk factors for the progression of open-angle glaucoma. *Arch Ophthalmol;* 126:1030-1036.

Chen QM, Liu J, Merret JB. (2000). Apoptosis or senescence-like growth arrest: influence of cell-cycle position, p53, p21 and bax in H2O2 response of normal human fibroblasts. *Biochem J* 347: 543-551

Copin B, Brézin AP, Valtot F, Dascotte JC, Béchetoille A, Garchon HJ. (2002) Apolipoprotein E-promoter single-nucleotide polymorphisms affect the phenotype of primary open-angle glaucoma and demonstrate interaction with the myocilin gene. *Am J Hum Genet*;70(6):1575-81.

Cordeiro F, Guo L, Coxon KM, Duggan J, Nizari S, Normando EM, Sensi SL, Sillito AM, Fitzke FW, Salt TE,E Moss SE. (2010). Imaging multiple phases of

neurodegeneration: a novel approach to assessing cell death in vivo. *Cell Death and Disease.*

Dean JL, Thangavel C, McClendon AK, Reed CA, Knudsen ES. (2010) Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. *Oncogene*; 29:4018-4032

Efeyan A, Garcia-Cao I, Herranz D, Velasco-Miguel S, Serrano M. (2006) Tumour biology: Policing of oncogene activity by p53. *Nature;* 443(7108):159

Fernández-Martínez L, Letteboer S, Mardin CY, Weisschuh N, Gramer E, Weber BHF, Rautenstrauss B, Ferreira PA, Kruse FE, Reis A, Roepman R, Pasutto F. (2011) Evidence for RPGRIP1 gene as risk factor for primary open angle glaucoma. *Eur J Human Genetics* 19: 445–451

Gallego-Pinazo R, Zanón-Moreno V, Sanz S, Andrés V, Serrano M, García-Cao I, Pinazo-Durán MD. (2008) Biochemical characterization of the optic nerve in mice overexpressing the P53 gen. Oxidative stress assays. *Arch Soc Esp Oftalmol*; 83:105-111

Gallego-Pinazo R, Pinazo-Durán MD, Serrano M. (2010). The cell cycle and gene p53. An approach to molecular ophthalmology. *Arch Soc Esp Oftalmol*. 85:229-231.

Garaci FG, Cozzolino V, Nucci C, Gaudiello F, Ludovici A, Lupattelli T, Floris R, Simonetti G. (2008) Advances in neuroimaging of the visual pathways and their use in glaucoma. *Prog Brain Res*; 173:165-77.

García-Cao I, García-Cao M, Martín-Caballero J, Criado LM, Klatt P, Flores JM, Weill JC, Blasco MA, Serrano M. (2002). "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO* ; 21:6225-6235.

Gaasterland D, Tanishima T, Kuwabara T. (1978). Axoplasmic flow during chronic experimental glaucoma. 1. Light and electron microscopic studies of the monkey optic nervehead during development of glaucomatous cupping. *Invest Ophthalmol Vis Sci*; 17:838-846.

Guevara NV, Kim HS, Antonova EI, Chan L. (1999). The absence of p53 accelerates atherosclerosis by increasing cell proliferation in vivo. *Nat Med*; 5:335-339.

Golubnitschaja O & Flammer J. (2007). What are the biomarkers for glaucoma? Survery Ophthalmology; 52:S155-S161

Harada Ch, Guo X, Namekata K, Kimura A, Nakamura K, Tanaka K, Parada L, Harada T (2011) Glia- and neuron-specific functions of TrkB signalling during retinal degeneration and regeneration. *Nature Communications.*

Hernández MR, Wang N, Hanley NM, Neufeld AH. (1991) Localization of Collagen Types I and IV mRNAs in

Human Optic Nerve Head by In Situ Hybridization. Investigative Ophthalmol Vis Sci. 32:2169-2177

Ivanova T, Gómez-Escoda B, Hidalgo E, Ayté J. (2011) G1/S transcription and the DNA synthesis checkpoint: Common regulatory mechanisms. *Cell Cycle* 10: 912-915

Jeong B-S, Hu W, Belyi V , Rabadan R, Levine AJ. (2009) Differential levels of transcription of p53-regulated genes by the arginine/proline polymorphism: p53 with arginine at codon 72 favors apoptosis. *FASEB J.* 24: 1347-1353

Ju WK, Liu Q, Kim KY, Crowston JG, Lindsey JD, Agarwal N, Ellisman MH, Perkins GA, Weinreb RN (2007), Elevated hydrostatic pressure triggers mitochondrial fission

and decreases cellular ATP in differentiated RGC-5 cell. *Invest Ophthalmol Vis Sci.;* 48:2145-51.

Haga SB, Khoury MJ, Burke W (2003) Genomic profiling to promote a healthy lifestyle: not ready for prime time. *Nat Genet* 34:347–350.

Khalyfa A, Chlon T, Qiang H, Agarwal N, Cooper NG. (2007) Microarray reveals complement components are regulated in the serum-deprived rat retinal ganglion cell line. *Mol Vis*; 13:293-308.

Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*; 26:239-57.

Kumarasamy NA, Lam FS, Wang AL, Teoharides TC. (2006) Glaucoma: Current and developing concepts in inflammation, pathogenesis and treatment. *Eur J Inflammation*; 4:129-137

Itoa Y, Shimazawaa M, Chenc YN, Tsurumaa K, Yamashimad T, Araiec M, Haraa H. (2009) Morphological changes in the visual pathway induced by experimental glaucoma in Japanese monkeys Exp Eye Res; 89:246-255

LaGrange, F, Beauvieux, J (1925). Anatomie de I'excavation glaucomateuse, *Arch Opthalmol (Paris)* 42:129.

Lam DS, Leung YF, Chua JK, Baum L, Fan DS, Choy KW, Pang CP. Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2000: 41:1386-91.

Lane DP (1992) p53, guardian of the genome. *Nature;* 358:15-16.

Lee KM, Miklos I, Du HY, Watt S, Szilagyi Z, Saiz JE, Madabhushi R, Penkett CJ, Sipiczki M, Bahler J, Fisher R P (2005) Impairment of the TFIIH-associated CDK-activating kinase selectively affects cell cycle-regulated gene expression I in fission yeast. *Mol Biol Cell* 16:2734 – 2745

Lee EJ, Kim TW, Weinreb RN, Park KH, Kim SH, Kim DM. (2011). {beta}-zone Parapapillary Atrophy and the Rate of Retinal Nerve Fiber Layer Thinning in Glaucoma. *Invest Ophthalmol Vis Sci.* 2011

Levin LA, Louhab AB (1996). Apoptosis of retinal ganglion cells in Anterior Ischaemic Optic Neuropathy. *Arch Ophthalmol;* 114:488-491

Levin, LA (1997). Mechanisms of optic neuropathy . *Curr Opin Ophthalmol* ; 78:9-15

Levin LA (1999) . Intrinsic survival mechanisms for retinal ganglion cells. *Eur J Ophthalmol*; 9 Suppl 1:S12-6.

Levine AJ, Finlay (2004) CA. P53 is a tumour suppressor gene. *Cell*; S116: 67-69

Levkovitch-Verbin H, Dardik R, Vander S, Nisgav Y, Kalev-Landoy M, Melamed S. (2006) Experimental Glaucoma and Optic Nerve Transection Induce Simultaneous Upregulation of Proapoptotic and Prosurvival Genes. *Invest Ophthalmol Vis Sci* 47: 2491-2497

Lopez-Martinez F, Lopez-Garrido MP, Sanchez-Sanchez F, Campos-Mollo E, Coca-Prados M, Escribano J (2007) Role of MYOC and OPTN sequence variations in Spanish patients with primary open-angle glaucoma. *Mol Vis*; 13:862-72.

Lutjen-Drecoll E, Shimizu T, Rohrbach M, Rohen JW (1986). Quantitative analysis of 'plaque material' in the inner- and outer wall of Schlemm's canal in normal- and glaucomatous eyes. *Exp Eye Res*; 42:443-55.

McKinnon SJ (1997); Glaucoma, apoptosis, and neuroprotection. *Curr Opin Ophthalmol*; 8:28-37

Meagher LC, Savill JS, Baker A, Fuller RW, Haslett C. (1992) Phagocytosis of apoptotic neutrophils does not induce macrophage release of thromboxane B2. *J Leuk Biol ;* 52: 269–272

Monemi S, Spaeth G, DaSilva A, Popinchalk S, Ilitchev E, Liebmann J, Ritch R, Héon E, Crick RP, Child A, Sarfarazi M. (2005). Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. *Hum Mol Genet* 14:725-733.

Muller H (1858). Anatomische Beitrge zur Qphthalmologie: Ueber Nervean-Veranderungen an der Eintrittsstelle'des Schnerven. *Arch Ophthalmol*; 41:1.

Nickells RW. (1999) Apoptosis of the retinal ganglion cells in glaucoma: an update of the molecular pathways involved in cell death. *Surv Ophthalmol* 43: S151-161

Osborne N, Wood J, Chidlow G, Bae J, Melena J, Nash N, (1999) Ganglion cell death in glaucoma: what do we really know?, *Br J Ophthalmol*.; 83: 980-986.

Osborne NN. (2010) Mitochondria: Their role in ganglion cell death and survival in primary open angle glaucoma. *Exp Eye Res*; 90:750-757.

Parisi V. (1997) Neural conduction in the visual pathways in ocular hypertension and glaucoma. *Graefes Arch Clin Exp Ophthalmol*.; 235:136-142.

Pease ME, McKinnon SJ, Quigley HA, Kerrigan-Baumrind LA, Zack DJ. (2000) Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. *Invest Ophthalmol Vis Sci*.; 41:764-774.

Pinazo-Durán MD, Renau Piqueras J, Guerri C (1993). Developmental changes in the optic nerve related to ethanol consumption in pregnant rats. Analysis of the ethanol-exposed optic nerve. *Teratology* 48:305-322.

Pinazo-Durán, M.D., Vallés, S., Guerri, C., Renau- Piqueras, J. (1996). Gestational-ethanol-induced changes in the developing optic nerve. *Int J Dev Biol*; Suppl 1:141S-142S.

Pinazo-Durán MD, Renau Piqueras J, Guerri C, Strömland K (1997) Optic nerve Hypoplasia in fetal alcohol syndrome: An update. *Eur. J. Ophthalmol*.; 50:100-111.

Pinazo-Durán MD, Gallego-Pinazo R, Pons Vazquez S, Muñoz A. (2005) Postnatal thyroid hormone supplementation rescues the retinal abnormalities in a rat model of congenital neonatal hypothyroidism. *Ophthalmic Res* ; 37: 225-234

Pinazo-Durán MD, Pons-Vázquez S, Gallego-Pinazo R, Galbis Estrada C, Zanón-Moreno V, Vila-Bou V, Sanz-Solana P. (2011) Thyroid hormone deficiency disrupts rat eye neurodevelopment. *Brain Res* .

Quigley HA, McKinnon SJ, Zack DJ, Pease ME, Kerrigan-Baumrind LA, Kerrigan DF, Mitchell RS, (2000) "Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats", *Invest Ophthalmol Vis Sci*.; 41:3460-6.

Quigley HA, Broman AT. (2006) The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*.; 90: 262–267.

Quiles-Perez R, Muñoz-Gámez JA, Ruiz-Extremera A, O'Valle F, Sanjuán-Nuñez L, Martín-Alvarez AB, Martín-Oliva D, Caballero T, Muñoz de Rueda P, León J, Gonzalez R, Muntané J, Oliver FJ, Salmerón J. (2010) Inhibition of poly adenosine diphosphate-ribose polymerase decreases hepatocellular carcinoma growth by modulation of tumor-related gene expression. *Hepatology*. ; 51:255-266.

Ray K, Mookherjee S. (2009) Molecular complexity of primary open angle glaucoma. Current concepts. *J. Genetics* ; 88: 451-467.

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Heon E, Krupin T, Ritch R, Kreutzer D, Crick RP, Sarfarazi M (2002) Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science 295:1077-1079.

Schori H, Kipnis J, Yoles E et al., (2001) Vaccination for protection of retinal ganglion cells against death from glutamate citotoxicity and ocular hypertension: implications for glaucoma. *Proc Natl Acad Sci USA*; 98:3398-3403

Schnabel, J (1892). Das glaucomatose Sehnervenleiden. *Archiv fur Augenheikunde*; XXIV: 18.

Serrano M, Hannon GJ, Beach D. (1993) A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704–707.

Snider WD. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* ; 77:627-638

Sitorus R Ardjo M, Lorenz B, Preising M. (2003) CYP1B1 gene analysis in primary congenital glaucoma in Indonesian and European patients. *J Med Genet* 40: 1-6

Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, et al. (1997) Identification of a gene that causes primary open angle glaucoma. *Science.* 275:668–70.

Tatton WG, Chalmers-Redman RM, Tatton NA. (2011) Apoptosis and anti-apoptosis signalling in glaucomatous retinopathy. *Eur J Ophthalmol. Suppl* 2:S12-22.

Trivino A, Ramirez JM, Salazar JJ, Ramirez AI, Garcia-Sanchez J. (1996). Immunohistochemical study of human optic nerve head astroglia. Vision Res; 36:2015–2028

von Jaeger E Ueber Glaucom und seine Heilung durch Iridectomie. *Z Ges der Aerzte zu Wien* 1858; 14:465- 484.

Walker NI, Harmon BV, Gobé GC, Kerr JF (1974) Patterns of cell death. *Methods Achiev Exp Pathol*. 1988; 13:18-54.

Wyllie AH. Death in normal and neoplastic cells. *J Clin Pathol Suppl*.; 7:35-42.

Yanagi M, Kawasaki R, Wang JJ, Wong TY, Crowston J, Kiuchi Y. (2010) Vascular risk factors in glaucoma: a review. *Clin Exp Ophthalmol*

Zanón-Moreno V, Pinazo-Durán MD. Impact of biomarkers in primary open-angle glaucoma. *Arch Soc Esp Oftalmol* 2008; 83: 465-468

Zanon-Moreno V, Garcia-Medina JJ, Zanon-Viguer V, Moreno-Nadal MA, Pinazo-Duran MD. (2009) Smoking, an additional risk factor in elder women with primary open-angle glaucoma. *Mol Vis.*; 15:2953-2959.

Zanón-Moreno VC, Pinazo-Durán MD. (2008) Impact of biomarkers in primary open-angle glaucoma. *Arch Soc Esp Oftalmol.*; 83:465-8

Zanon-Moreno V, Marco-Ventura P, Lleo-Perez A, Pons-Vazquez S, Garcia-Medina JJ, Vinuesa-Silva I, Moreno-Nadal MA, Pinazo-Duran MD. (2008) Oxidative stress in primary open-angle glaucoma. *J Glaucoma.*; 17:263-268.

Zhang Y, Bhavnani BR. Glutamate-induced apoptosis in primary cortical neurons is inhibited by equine estrogens via down-regulation of caspase-3 and prevention of mitochondrial cytochrome c release. *BMC Neurosci.* 2005; 6:13.

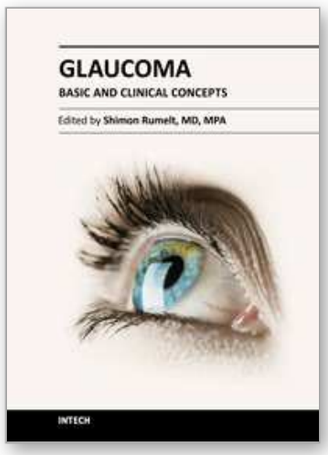
Virág L (2005) Structure and function of poly(ADP-ribose) polymerase-1: role in oxidative stress-related pathologies. *Curr Vasc Pharmacol.*; 3:209-214.

Zeisel SH (2007) Nutrigenomics and metabolomics will change clinical nutrition and public health practice: insights from studies on dietary requirements for choline. *Am J Clin Nutr* 86:542–548

Zhong L, Bradley J, Schubert W, Ahmed E, Adamis AP, Shima DT, Robinson GS, Ng YS. (2007) Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol Vis Sci*. 48:1212-1218.

IntechOpen

IntechOpen



Glaucoma - Basic and Clinical Concepts
Edited by Dr Shimon Rumelt

ISBN 978-953-307-591-4
Hard cover, 590 pages
Publisher InTech
Published online 11, November, 2011
Published in print edition November, 2011

This book addresses the basic and clinical science of glaucomas, a group of diseases that affect the optic nerve and visual fields and is usually accompanied by increased intraocular pressure. The book incorporates the latest development as well as future perspectives in glaucoma, since it has expedited publication. It is aimed for specialists in glaucoma, researchers, general ophthalmologists and trainees to increase knowledge and encourage further progress in understanding and managing these complicated diseases.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Maria D. Pinazo-Durán, Roberto Gallego-Pinazo, Vicente Zanón-Moreno and Manuel Serrano (2011). Glaucoma Genetics – Regulation of Cell Surviving and Death in the Retina, Glaucoma - Basic and Clinical Concepts, Dr Shimon Rumelt (Ed.), ISBN: 978-953-307-591-4, InTech, Available from: http://www.intechopen.com/books/glaucoma-basic-and-clinical-concepts/glaucoma-genetics-regulation-of-cell-surviving-and-death-in-the-retina

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen