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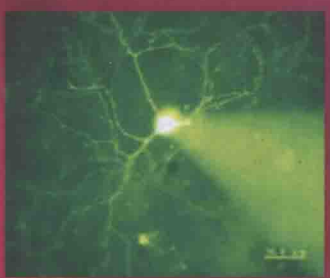

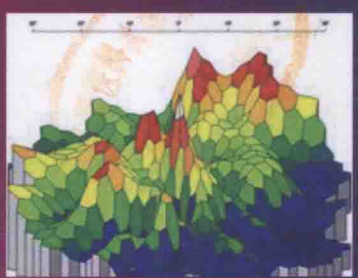

Eye and Vision Research Developments Series

RETINITIS PIGMENTOSA

Causes, Diagnosis and Treatment

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EYE AND VISION RESEARCH DEVELOPMENTS SERIES

RETINITIS PIGMENTOSA: CAUSES, DIAGNOSIS AND TREATMENT

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Nova Biomedical Books
New York

Library of Congress Cataloging-in-Publication Data

Retinitis pigmentosa : causes, diagnosis, and treatment / editors, Michaël Baert and Cédric Peeters.

p. ; cm.

Includes bibliographical references and index.

ISBN 978-1-60876-884-4 (softcover : alk. paper)

1. Retinitis pigmentosa. I. Baert, Michaël. II. Peeters, Cédric.

[DNLM: 1. Retinitis Pigmentosa--etiology. 2. Retinitis Pigmentosa--diagnosis. 3. Retinitis Pigmentosa--therapy. WW 270 R438895 2009]

RE661.R45R467 2009

617.7'35--dc22

2009045932

Published by Nova Science Publishers, Inc. † New York

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Preface

Retinis Pigmentosa (RP) includes a group of progressive hereditary retinal diseases involving degeneration of rod and cone photoreceptors, predominantly the former, and is one of the leading causes of hereditary blindness in the developed world. Clinical symptoms include nyctalopia, progressive visual field loss, and deterioration in visual acuity in adolescence. It affects one in 3000-5000 individuals and can be caused by mutations in more than 40 genes. In addition, Retinitis Pigmentosa may exist either alone (nonsyndromic) or as part of a neurological or systemic disorder, such as Usher's syndrome and Infantile Refsum's disease. There are few effective clinical treatments for retinitis pigmentosa which affects an estimated 1.5 million individuals worldwide. However, understanding the histopathologic changes occurring in RP is critical to understanding the rationale for current therapies, as well as to develop future therapies. This book highlights the most recent research done in the field.

Chapter I - Retinitis Pigmentosa is one of the leading causes of blindness worldwide and lacks effective clinical treatment. Stem cell-based therapy offers a novel experimental therapeutic approach, based on the strategy that transplanted progenitor cells could replace damaged photoreceptor cells. However, it is still unknown what is the optimal time to choose for targeting the host tissue during the progression of the degeneration, the characteristics and potential capacities in different stem cells, whether stem cells differentiate into functional daughter cells, and the degree to which host retinal function can be restored.

We have used Royal College of Surgeons (RCS) rats as a suitable model of retinitis pigmentosa and light induced photorecptor damage in minipigs to

study the effectiveness of cell transplant therapies and the functional capcity of the retina. Initially, whole-cell patch clamp studies showed three action potential discharge patterns of retinal ganglion cells (RGCs) in RCS rats: single, transient, and sustained firing. The main discharge pattern was single firing between postnatal weeks 1 and 2 (P1-2W), followed later by transient and sustained firing patterns. However, during later stages of retinal degeneration at P7-8W, 26.7% RGCs lack action potentials in RCS rats, and this proportion had increased (63.2% of RGCs) by P9-12W. This suggested functional RGCs were maintained in the early stages of retinal degeneration, but this functional parameter was lost during retinal degeneration, even though morphological differences are not apparent. Cultured stem cells from embryonic rat retina differentiated and produced action potentials in vitro showing that the maturation of electrophysiological properties of presumptive RGCs occurs after at least 15 days under culture conditions. Knowledge of the timing of voltage-dependent ion channel development provides a time window stem cell funcitonal matureation that will help to improve success rate in transplantation protocols if functional recouvery is to be achieved. We found that, three types of stem cells (rat optic cup at embryonic day 12.5 (OC-RSCs), retinal stem cells from embryonic day 17 induced by BDNF (RSCs–BDNF) and rat bone marrow stromal cells (BMSCs)) were incorporated into the degenerating retina and differentiated into rhodopsin positive cells. Thus, all three stem cell types are suitable for retinal transplantation, although RSCs from different developmental stages have distinct proliferative capacities and differentiation potential. OC-RSCs are easier to passage, although a large number of E12.5 embryos are required. BMSCs are easier to obtain and can restore retinal function in RCS rats for up to two months after transplantation. The retinal transplantation in minipigs by neonatal pig retina or human fetal retina with an intact retinal pigment epithelium (RPE) was successful in 66.7% of the experiments. Twelve months follow up showed that xenografts transplantation had not resulted in any immunological rejection and thus was a safe technique. After the human fetal retina transplantation, the grafts survived and retained characteristics of progenitor precursor cells, such as Chx10 labeling. Multifocal electroretinography (mfERG) showed improvement of central posterior retinal function from the 1st to 8th month after transplantation. Müller cells are an integral and important glial component in the normal function of the retina and form an vital part of the regenertive process. Immunocytochemistry showed that Müller cells express retinal progenitor cell markers during chronic retina degeneration. After RSC transplantation, Müller

cells were seen to differentiate into photoreceptors within and nearby the grafted area. This suggests that Müller cells have the potential to re-enter the cell cycle and can differentiate into host photoreceptor cells thus restoring lost retinal components. Further studies are needed to determine how to maintain the progenitor potential of Müller cells and how RCS transplants may augment this process of restoring cells, especially photoreceptors, to the damaged retina. These studies may help to understand how host retinal function can be restored with these combined techniques.

Chapter II - Retinis Pigmentosa (RP) is a term that includes a group of progressive hereditary retinal diseases involving degeneration of rod and cone photoreceptors, predominantly the former, and is one of the leading causes of hereditary blindness in the developed world. Ganglion cells are also affected, possibly due to transsynaptic neuronal damage caused by loss of neuronal input from the degenerating photoreceptor cell layer. Clinical symptoms include nyctalopia, progressive visual field loss, and deterioration in visual acuity in adolescence. No effective therapy exists at present. It affects one in 3000-5000 individuals and can be caused by mutations in more than 40 genes. Retinitis Pigmentosa may exist either alone (nonsyndromic) or as part of a neurological or systemic disorder, such as Usher's syndrome and Infantile Refsum's disease. Typical findings on retinal examination include retinal vessel attenuation. Bone spicule formation is also noted around the intraretinal vessels (pigmentary clumping), caused by the hyperplasia and migration of retinal pigment epithelial cells into the inner layers of the neurosensory retina. The disease may be inherited as either autosomal recessive, autosomal dominant or X-linked. Autosomal dominant inheritance is the most common. Several mechanisms to explain the degeneration process have been proposed. These include misfolding of the rod visual pigment Rhodopsin preventing its transportation to the outer segment, dysfunction of cell transport systems that are involved with photoreceptor protein localization, starvation of cones and cyclic GMP-dependent protein kinase activation. Therapies that are currently under investigation include the use of ribozymes for the targeted reduction of mutant-allele mRNA and the use of retinoids to improve protein folding.

Chapter III - Recently, *RPE65* gene replacement therapy in a total of nine human subjects with Leber congenital amaurosis marked the first treatment for genetic retinal degenerative diseases (RDD). The prospect of imminent gene therapy made *RPE65* a strong candidate for mutation screening in South Africa. An added impetus for this study was the fact that a founder mutation in *RPE65* was described as causing an early onset RDD in an isolated Dutch

population, and the founder effect in South Africans descended from Dutch settlers has been well documented for other diseases and genes.

Mutations in the *RPE65* gene are reported to be responsible for approximately 2% of autosomal recessive retinitis pigmentosa (arRP) and 16% of Leber congenital amaurosis (LCA). For this study a cohort of 87 affected, unrelated individuals was selected for mutation screening. Of these individuals, 18 were classified as having LCA and 69 as having early onset RP (with an age of onset younger than 15 years). Of the LCA cohort, 4 exhibited autosomal recessive inheritance (arLCA) and 14 were isolated cases. Of the RP cohort, 44 had arRP and 25 were isolated cases. The ethnic breakdown of the cohort was as follows: 65 were Caucasian, 7 were indigenous Black African, 10 of Asian Indian origin, 4 of Mixed Ancestry (comprising individuals whose ancestry is a mixture of Caucasian, Malaysian, Madagascan and indigenous African including Khoi-San and West African) and 1 was Taiwanese. The 14 exons of *RPE65*, including the intron/exon boundaries, were screened using denaturing high performance liquid chromatography (dHPLC) analysis and variations were characterised by direct sequencing.

Five different pathogenic mutations (of which 2 were novel) were identified in the cohort of 69 individuals diagnosed with early onset RP. The Dutch founder mutation, Tyr368His, was the only homozygous mutation detected and was identified in a family of Mixed Ancestry with arRP. In two families, compound heterozygous mutations in *RPE65* are presumed to be causative of disease: Ala132Thr and the novel IVS1+1G>T mutation was present in one Indian family with arRP; Leu22Pro and the novel Glu21Lys mutation were present in one Caucasian individual with isolated RP. In two cases (one Indian family with arRP and one Caucasian individual with isolated RP) a single heterozygous Ala132Thr mutation was identified and the second mutation, should it exist, is unknown. This Ala132Thr mutation was the single most common variation detected, as it was identified in three of the 69 individuals classified as having RP (4.4%). No pathogenic mutations were identified in the cohort of patients diagnosed with LCA.

The identification of disease-causing genetic mutations in families with RDD generally means that predictive, diagnostic and prenatal testing can be offered to family members, although few options exist for treatment. Importantly, three families in South Africa possibly stand to benefit from therapeutic intervention by *RPE65* gene replacement therapy.

Chapter IV - Retinitis pigmentosa (RP) is a group of inherited retinal degenerations, caused by mutations in one of many genes - some already identified, and some still yet to be discovered. While there are many different genes involved and great heterogeneity among these genes, the underlying common source of vision loss is retinal dysfunction related to photoreceptor loss. The sequence of histopathologic changes associated with RP occurs in several stages. The 1st stage is associated with rod photoreceptor dysfunction and ultimate death and the second with cone photoreceptor demise. Following photoreceptor loss, a number of secondary changes occur, with retinal pigment epithelial (RPE) cells detaching from of Bruch's membrane and migrating into the inner retina to ultimately accumulate and surround blood vessels which gives rise to the "bone spicules" observed clinically. Other pathologies include attenuation of blood vessels, retinal gliosis, migration of microglia into the outer retina, optic nerve atrophy and mild vitritis. Current therapies for RP include: genetic replacement of missing/mutated proteins via viral vectors, addition (by injection or surgery) of factors or supplements that may prolong photoreceptor survival, transplantation of photoreceptors and RPE cells and electrical stimulation of remaining neurons, as well as developing therapies targeted at preventing photoreceptor apoptosis. Understanding the histopathologic changes occurring in RP is critical to understanding the rationale for current therapies, as well as to develop future therapies. Mouse models of retinitis pigmentosa, have been instrumental in aiding the study of histopathologic changes that occur in the setting of retinitis pigmentosa and to initiate and study various treatment approaches. Many of the findings of the histopathologic changes and treatment avenues are explored in these models.

Chapter V - We identified mutations in the RHO and RDS genes in patients with autosomal dominant and sporadic forms of retinitis pigmentosa (RP) from Volga-Ural region of Russia. The 5 exons of RHO and 3 exons of RDS genes were analyzed for sequence changes by single-strand conformation polymorphism (SSCP) and direct sequencing. Patients were examined clinically and with visual function tests. We detected known mutation Pro347Leu and novel mutation Arg252Pro and two polymorphisms IVS1+10g>a, IVS3+4c>t in RHO gene. There were statistically significant differences in allele and genotype frequencies of sequence change IVS3+4c>t of RHO gene in affected patients with RP and in controls. According to our data, this polymorphism is likely to be pathogenic. Recently were reported 16 possible combinations of the exon 3 RDS gene SNPs and detected four from 16 possible combinations (minihaplotypes): $G^{1147}A^{1166}G^{1250}C^{1291}$ (I),

$C^{1147}A^{1166}A^{1250}C^{1291}$ (II), $C^{1147}G^{1166}A^{1250}C^{1291}$ (III) and $G^{1147}A^{1166}G^{1250}T^{1291}$ (IV). Telmer C.A. 2003 established that minihaplotype $C^{1147}A^{1166}A^{1250}C^{1291}$ (II) was linked to the mutation IVS2+3a>t. Sequencing results for variant positions in exon 3 RDS gene in RP patients from Volga-Ural region showed all four minihaplotypes described above and minihaplotype $C^{1147}A^{1166}A^{1250}T^{1291}$ (V). Our study determined that minihaplotype $C^{1147}A^{1166}A^{1250}C^{1291}$ (II) isn't linked to mutation IVS2+3a>t in adRP and sporadic RP patients from Volga-Ural region. To optimize the DNA diagnostics of retinitis pigmentosa, it is necessary to analyze patients from various ethnic groups. Our study helps in molecular characterization of RP in Russia.

Chapter VI - The rd1 (retinal degeneration) mouse retina shows degeneration homologous to a form of retinitis pigmentosa with a rapid loss of rod photoreceptors and deficiency of retinal blood vessels. Due to Pde6brd1 gene mutation, β subunit of phosphodiesterase (PDE) of rd1 retina has an inactive PDE which elevates cGMP and Ca2+ ions level. In vitro retinal explants provide a system close to the in vivo situation, so both approaches were used to compare the status of oxidative stress, transforming growth factor-β1

(TGF-β1), sialylation, galactosylation of proteoglycans, and different proteinases-endogenous inhibitors systems participating in extracellular matrix (ECM) remodeling/degeneration and programmed cell death (PCD)/apoptosis in wt and rd1 mouse retinas.

Proteins and desialylated sulfated glucosaminoglycan parts of proteoglycans in ECM of rd1 retina were, respectively, decreased and increased due to enhanced activities of proteinases. Desialylation increases the susceptibility of cells to phoagocytosis/ apoptosis, decreased neurogenesis and faulty guidance cues for synaptogenesis. In vivo activities of total proteinases, matrix metalloproteinase-9 (MMP-9) and cathepsin B were increased in rd1 retina on postnatal day 14 (PN14), -21 and -28, due to relatively lower levels of tissue inhibitor of MMPs (TIMP-1) and cystatin C, respectively. This corresponded with increased in vitro secretion of these proteinases by rd1 retina. Cells including end-feet of Mueller cells in degenerating rd1 retina showed intense immunolabeling for MMP-9, MMP-2/TIMP-1, TIMP-2 and cathepsin B/cystatin C, and proteinases pool was increased by Mueller cells. Intense immunolabeling of ganglion cell (RGC) layer for cathepsin B and of inner-plexiform layer of both PN2/PN7 rd1 and wt retinas indicated importance of cathepsin B in synaptogenesis and PCD of RGC.

Increased levels of TGF-β1 in vitro transiently increased the secretion of MMPs and cathepsins activities by wt explants which activate TGF-β1 and remodel the ECM for angiogenesis and ontogenetic PCD. Whereas, lower level of TGF-β1 and persistently higher activities of MMPs and cathepsins in rd1 retinas and conditioned medium, suggested that proteinases degraded TGF-β1 and ECM and caused retinal degeneration.

Lower activities of glutathione-S-transferase and glutathione-peroxidase in rd1 retina contribute to oxidative stress which damages membranes and increased the expression, release/secretion of proteinases relative to their endogenous inhibitors. Participation of oxidative stress in rd1 retinal degeneration was further confirmed from the partial protection of rd1 photoreceptors by in vitro and/or in vivo supplementation with glutathione-S-transferase or a combination of antioxidants namely lutein, zeaxanthin, α-lipoic acid and reduced-L-glutathione. Treatment with combination(s) of broad spectrum proteinase inhibitor(s) and antioxidants needs investigation.

Chapter VII - The aim of our study was to ascertain if visual training by means of Visual Pathfinder (LACE inc.) biofeedback system could be successful to improve and/or restore visual function in visually impaired patients with retinitis pigmentosa.

We enrolled 15 patients (age range 8-55) and examined a total of 30 eyes with retinitis pigmentosa. All the patients underwent a complete ophthalmologic evaluation which comprised the assessment of best corrected visual acuity (BCVA) and pattern reversal visual evoked potential (VEP) according to the ISCEV standards. All the patients underwent 10 training sessions of 10 minutes each eye performed once a week using the Visual Pathfinder.

Statistical analysis was performed using the Student's t-test. P values less than 0.05 were considered statistically significant.

The mean BCVA was 0.67± 0.14 logMAR at the baseline assessment, and 0.84± 0.11 logMAR at the end of visual training; this result was statistically significant (p=0.035). VEP amplitude of P100 wave was 2.14±0.88 mV at the baseline assessment, and 4.86 ±1.12 mV at the end of visual training; this result was statistically significant (p=0.012).

In conclusion our experience demonstrates that visual training by means of a visual evoked acoustic biofeedback with Visual Pathfinder can significantly improve visual acuity and pattern reversal VEP amplitude in retinitis pigmentosa, resulting in more suitable visual performances, better

quality of life, and a much more positive psychological situation for these patients.

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Chapter I

Experimental therapy for Retinitis Pigmentosa

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Abstract

Retinitis Pigmentosa is one of the leading causes of blindness worldwide and lacks effective clinical treatment. Stem cell-based therapy offers a novel experimental therapeutic approach, based on the strategy that transplanted progenitor cells could replace damaged photoreceptor cells. However, it is still unknown what is the optimal time to choose for targeting the host tissue during the progression of the degeneration, the characteristics and potential capacities in different stem cells, whether stem cells differentiate into functional daughter cells, and the degree to which host retinal function can be restored.

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