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Review

Progress in histopathologic and pathogenetic research in a retinitis pigmentosa model

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Summary. Retinitis pigmentosa is a major cause of visual impairment and blindness, affecting millions of people worldwide. The mechanisms of and effective treatments for the disease, however, remain to be further investigated. The Royal College of Surgeons rat is one of the most widely used animal models for the study of retinal degeneration diseases. The mutation in the *mer tyrosine kinase* proto-oncogene of this model leads to deficient phagocytosis in the retinal pigment epithelium cells and the accumulation of photoreceptor out segments in the subretinal space, ultimately resulting in retinal degeneration. The retina begins to change as early as 17 days after birth and becomes gradually thinner with the death and remodeling of cells and blood vessels. Retinal cell apoptosis plays a dominant role in this degeneration, with some cells being activated by the secondary alterations of the retinal neurotransmitter and other related factors.

Key words: Retinitis pigmentosa, Royal College of surgeons rat, Histopathology, Mechanism

Introduction

Retinitis pigmentosa (RP) is a major cause of visual impairment and blindness, affecting millions of people worldwide. This heterogeneous group of disorders, all affecting the functions of the retinal pigment epithelium (RPE) and the photoreceptors, eventually results in a loss and remodeling of the neurons and vasculature. These genetic changes have been extensively studied, and mutations in nearly 100 genes have been found, including autosomal recessive, autosomal dominant and X-linked modes of inheritance (Xie et al., 2014). However, the mechanisms of the disease and effective treatments remain to be further investigated. The Royal College of Surgeons (RCS) rat is one of the most widely used animal models for the study of retinal degeneration and was first described as a cataract and spontaneous RP model in 1938 (Bourne et al., 1938a,b). The most important gene in this model is the mer tyrosine kinase (*MERTK*) proto-oncogene, which encodes tyrosine kinase receptors and is expressed in RPE cells (Gal et al., 2000). Phagocytosis deficiency leads to the accumulation of photoreceptor out segments (OS) in the subretinal space, ultimately resulting in retinal degeneration (Mullen and LaVail, 1976). Because some patients with photoreceptor degeneration have the mutations in the same gene, the RCS rat can be considered a useful model for mechanistic and therapeutic studies. Thus, identifying the changes involved in retinal degeneration will improve our knowledge of the mechanisms and potential treatments for RP. In the present review, we summarize the changes observed in RCS rats and the current knowledge of the related mechanisms.

Morphological changes of the RCS rat retina

The retinas of RCS rats become gradually thinner during the degenerative process. In normal mature rats, 12-14 rows of photoreceptor cell nuclei can be seen in the outer nuclear layer (ONL) and five rows in the inner nuclear layer (INL). In the retinas of the RCS rats, the ONL is reduced to 10 rows and the INL is 5 cells deep at

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postnatal (P) 22 days (d). The ONL thickness decreases significantly, even to a single layer, in a 3-month-old RCS retina. The OPL is also very thin, whereas the INL is still regularly arrayed at a normal thickness. At 8 months, the INL becomes uneven, with a thickness of 1 to 10 cells, and the cells migrate into the inner retina as RPE cells migrate from Bruch's membrane (Girman et al., 2005; Wang et al., 2005). In the RCS rat, the most prominent characteristic of retinal dystrophy is an OS debris zone that is present from an early stage, persisting at P55d but missing from the posterior half of the retina (Pennesi et al., 2008).

Retinal cell changes

Photoreceptor cells

Retinal degeneration begins with an alteration of the rod OS at P17d (Armata et al., 2006) and the thickening of the ONL at P22d (Tso et al., 1994), as RPE cells lose their ability to phagocytose rod OS (D'Cruz et al., 2000). Still, obvious rod apoptosis cannot be observed until P25d. By P35d, the ONL thickness has significantly decreased, corresponding with the peak of rod degeneration. Most of the rods can be characterized as either dead or degenerated by P90d (Peng et al., 2003).

Cone development is both morphologically and functionally complete by approximately P21d in both RCS and control rats. At P30d, the OS have become shorter and the inner segments (IS) larger, indicating the obvious degeneration of cones. The number of cones has been shown to decrease gradually (Peng et al., 2003). Most of the OS are lost by P45d, and the remaining IS are more swollen. In addition, the IS density declines more slowly in the peripheral, nasal, and superior retina. By P60d, IS cannot be seen in the central retina and appear only peripherally. At P90d, the cones have become distorted and few cone pedicles can be identified (Huang et al., 2011).

Bipolar cells

In normal rats, rod bipolar cells band a rod with a dendritic arbor, with a single axon passing through the inner plexiform layer (IPL) perpendicularly and ending near the IPL and retinal ganglion cell (RGC) layer border with terminal bulbs (Wang et al., 2005). By P22d in RCS rats, the rod bipolar cells have begun to exhibit smaller terminal end-bulbs, fewer dendrites, and shorter axons than controls. At 2 months of age, the dendrites are no longer erect but have become flattened and the axonal terminals have become smaller. By 3 months of age, the dendrites have grown into the debris zone and the end bulbs are smaller and less complex. From 5 to 18 months, the dendrites continue to diminish, the axons continue to retract and the axon terminals become fewer in number. These changes are most severe in the ventral retina (Wang et al., 2005; Armata et al., 2006). On different postnatal days, the number of remaining rod bipolar cell bodies is always greater than that of rod photoreceptors (Peng et al., 2003).

Two types of cone bipolar cells have been identified, including the smaller ON cone bipolar cells, the axons of which end in the inner IPL, and the larger OFF cone bipolar cells, the axons of which end in the outer IPL (Hartveit, 1997). In normal rat retinas, both types of bipolar cells are interspersed in the outer part of the INL in one or two rows. In dystrophic RCS retinas, there are no significant changes except for the inward movement of some cell bodies to the INL until 2 months of age. By 3 months, the cone bipolar cells have finished moving toward the photoreceptors. The OFF cone bipolar cells continue moving to the middle part of the INL, whereas the loss of ON cone bipolar cells has become considerable and is more severe in the peripheral retinal regions with vanished axon terminals. The surviving ON bipolar cells remain immature and have no normal electrophysiological characteristics (Zhang et al., 2010). At 5 months, most of the bipolar cells remaining are of the OFF type and their axonal terminals are decreased. At 8 months, the cells are disrupted by deformed blood vessels, with no cone bipolar cells being found in some areas, and the far-fewer axonal terminals have distorted structures (Wang et al., 2005).

The synaptic connectivity between photoreceptors and the processes of bipolar and horizontal cells have already been impaired before substantial photoreceptor loss is observed and become progressively diminished, losing their presynaptic-postsynaptic paired appearance and eventually becoming irregularly distributed foci (Pu et al., 2006). In addition, abnormal synaptic contacts begin to form. Rod bipolar cell dendrites begin to synapse flat-contact at early stages, with proven abnormal synaptic contacts at both the cone and rod terminals, indicating a similar loss of selective cell connectivity between bipolar cell dendrites and photoreceptors as has been observed in other animal models of retinal dystrophy (Peng et al., 2003; Haverkamp et al., 2006).

Horizontal cells

Horizontal cells connect rods with axons and cones with dendrites and are connected to each other by gap junctions. Normally, their cell bodies are located in the outer INL, with dendrites extending radially from cell soma and axons forming a narrow plexus in the outer plexiform layer (OPL). In RCS rats, the cell processes show no obvious changes until P45d. By 2 months of age, the dendrites become small and flat, mixing with the cell bodies; the ratio of dendrite length to axon length is reduced. At the age of 3-6 months, the cells extend widely in depth, especially in the peripheral retina. The dendrites sprout into the subretinal space, and the axon terminals are no longer found in their horizontal linear structure, instead germinating toward the ONL and into the INL. From 8 months onward, a majority of the processes grow into the IPL and INL, but

in dwindling numbers. The disrupted cell bodies are associated with the blood vessels, and these changes can be seen throughout the retina (Wang et al., 2005; Pinilla et al., 2007).

Amacrine cells

Amacrine cells comprise the most varied group of cell types in the retina, among which the AII amacrine cell is one of the best characterized. The AII amacrine cells, the bodies of which reside in the INL and the processes of which sprout into the IPL toward the ganglion cell layer, have synapses among different types of bipolar cells and ganglion cells and play critical roles in the rod-driven circuit. In RCS rats, from 3 months onward, the processes of AII amacrine cells in the IPL are gradually reduced and distorted in most of the retinal area (Lee et al., 2004; Wang et al., 2005).

Another class of amacrine cells, which can be labeled by the calretinin antibody, shows three rows of bodies and terminals in the inner IPL in nondystrophic RCS rats. In dystrophic RCS rats, the terminals form thinner bands compared to controls from P22d, lasting for 4 months. By 5 months of age, the inner bands of the terminals move toward the ganglion cell layer. At later stages, the disruption of the cell processes and bodies can be seen over most areas of the retina (Wang et al., 2005).

Retinal ganglion cells

In RCS rats, there are no obvious changes in the RGC layer before 4 months. At 5 months, some axons can be observed to be budding into the IPL, showing distortions, dilatations and regeneration at the inner retinal vessels' cross-sites at 6 months and running into the RPE layer after 8 months, at which point some ganglion cell bodies become swollen and atrophic and exhibit decreased densities (Wang et al., 2003). Intraretinal dendritic transport and axonal regeneration have been suggested to be impaired with this diminution of photoreceptor sensory input (Marco-Gomariz et al., 2006). Axonal vascular compression has been proposed as a dominant pathogenetic mechanism of disease rather than a consequence of the dystrophy (Wang et al., 2005; Garcia-Ayuso et al., 2014).

Melanopsin cells

This type of intrinsically photosensitive retinal ganglion cell (ipRGC) is located in the inner region of the retina and generates signals to the brain for image formation and circadian rhythms (Dacey et al., 2005). At 15 weeks of age in RCS rats, the distinctive molecular structure of the ipRGCs remains unaltered. After 5 months of age, some melanopsin-positive fibers sprout into the remaining INL, distorting the dendrites; this process has been thought to be associated with abnormal vasculature. A considerable number of melanopsin-positive cells, however, survive to the later stages and show some signs of remodeling (Vugler et al., 2008).

Müller cells

At P15d, Müller cells span the entire thickness of the retina, with the cell bodies in the middle of the INL and branches within the OPL and the IPL (Zhao et al., 2010). Up to 1 month after birth, Müller cells are still able to react and proliferate (Kimura et al., 2000). The Müller processes become hypertrophic and extend into the debris zone by 3 months (Zhao et al., 2010). In areas without debris, sprouting is lost and can be seen only in the far peripheral regions, which still contain some debris after 5 months. In the later stages, a glial seal forms along the outer layer of the retina (Wang et al., 2005; Zhao et al., 2012).

Findings of the ocular fundus

Takeo and colleagues examined the changes in the ocular fundus (Satoh and Yamaguchi, 2000). In the fundus at 3 weeks of age, a reddish-colored background with well-developed arterioles and venules can be seen, although this region changes to a pale color at 7 weeks after birth before becoming paler at 9 weeks with attenuated blood vessels. In FFA, the background fluorescence becomes gradually brighter and is uniformly bright at 5 min after dye injection in the RCS rats at 3 weeks of age, similar to the control rats. Hyperfluorescent dye leakage appears on the bright background at 5 weeks of age and spreads over the dark background after 7 weeks of birth. This leakage seems to be related to the dysfunction in the tight junctions between RPE cells. After ICG injection, a bright background appears gradually at postnatal 3 weeks, although this effect can also be seen in the normal controls. By 5-9 weeks of age, hyperfluorescent lesions appear and increase in size and number over a dark background, possibly indicating the accumulation of subretinal debris and choriocapillary atrophy.

Intraretinal oxygen levels

The oxygen distribution in the retina is characterized by oxygen from the choroidal and retinal vascular systems and oxygen consumption in the inner retina. In normal mature rats, the oxygen level reaches a minimum in the IPL and outer retina but peaks in the choroid (Yu et al., 1994). There is no significant difference between the dystrophic RCS rats and the age-matched controls at P20d; however, changes are observed starting at approximately P30d. The high oxygen uptake in the outer retina is gradually reduced, although oxygen consumption is still evident in the inner regions, reflecting the loss of photoreceptor oxygen consumption and relatively normal oxygen uptake by the inner retina

after this loss of photoreceptors (Yu et al., 2000).

Blood vessels and blood flow

In addition to the degeneration of the photoreceptor cells, secondary changes in the vasculature have been described.

SD rat retinas show a well-organized vascular network throughout every period of life. In P29d RCS retinas, the capillaries are not fully developed, exhibiting caliber fluctuations and crosstalk between vessels and vessels with nearly no perfusion, especially in middle and peripheral retina (Prokosch et al., 2011). Among the two sets of RBV, the superficial plexus of the retinal vascular system in RCS rats develops normally and shows no obvious difference from the wild-type at 8 months of age (Pennesi et al., 2008). The deep capillary plexus, however, reduces quickly by 3 months of age when the debris zone is cleared, and the vessels remodel into dilated and folded vascular complexes (McKenzie et al., 2012). Hyperfluorescent spots and angiographic leakage begin to emerge at 10 weeks and become exacerbated with time (Zambarakji et al., 2006). The vessels course from the RGC layer to the RPE layer obliquely. Distorted cell bodies and processes can be found to migrate along the abnormal blood vessels, starting from the ventral of the optic nerve and gradually spreading to most regions of the retina (Wang et al., 2003, 2005). At the late stages of photoreceptor degeneration, neovascularization becomes progressively apparent throughout the retinal surface (Pennesi et al., 2008). In addition, the blood vessel density shows a substantial decrease that continues to at least 1 year of age (MT, 1985).

Retinal blood flow (RBF) decreases with progressive retinal degeneration. At P40d, the RBF of RCS rats develops normally, showing no significant difference from that of control rats (Li, et al., 2012a). Magnetic resonance imaging has been applied to this process and has shown that the basal blood flow of the whole retina in the adult RCS rat was 5 times lower than that in the normal controls, although the vascular reactivity was not impaired (Li et al., 2009; Nair et al., 2011). The choroid blood flow, however, does not change during the progression of the disease compared with age-matched normal controls (Li et al., 2012a).

What happens to the other regions of the RCS rats?

Although RCS rats have been used widely to study retinal degeneration, they were first used as a model of cataracts, specifically of posterior subcapsular cataracts (Bourne et al., 1938a). These rats also exhibit other changes not restricted to the retina and lens.

In the RCS iris, substance P nerve fibers increase with age, together with vasodilatation. The surface of the pars plana is covered by venules, whereas the sympathetic TH-positive nerve fiber supply decreases (May, 2011). Iris or ciliary body colobomas can be found in some RCS rats, mostly in the inferior regions associated with small-sized eyes, inferior ectopic pupils and severe chorioretinal colobomas. In the colobomal area, the iris is very narrow or absent and the ciliary body invaginates toward the optic disc from the periphery to the central part of the tissue defect, with the corneoscleral region curving along the ciliary body. The affected area consists of the sclera, fibrovascular tissue containing small blood vessels, a few larger arteries, and a muscular layer. The retinal layer is often completely or partially lost (Tsuji et al., 2011).

The visual cortex of RCS rats is not competent in responding to visual stimulation at 4 weeks of age, when the development has not yet been completed (Gias et al., 2011). At later stages, with the progressive loss of ganglion cells and reduced retinal input into the cortex, signs of fiber loss in the superior colliculus can be found at the age of 9 months and may further degrade by 19-26 months (Vugler and Coffey, 2003).

Mechanisms related to retinal degeneration

Photoreceptor and other retinal cells

The degeneration of photoreceptor cells is suggested to be mediated mainly by apoptosis, arising from the accumulation of retinal OS debris between photoreceptor cells and RPE (Goldman and O'Brien, 1978). Various signals initiate the apoptosis pathway, including oxidative stress and changes in the factors participating in cell survival and death.

At the initial stage of RCS rat retinal degeneration, the cytosolic and mitochondrial calpains are reported to initiate apoptosis, which occurs even earlier than the caspase pathways. Mitochondrial calpain is activated before cytosolic calpain, a process in which the μ-calpain precursor is translocated from the cytosol to the mitochondria, inducing apoptosis by activating the apoptosis-inducing factor (Mizukoshi et al., 2010). In another study, the calpains were found not to be involved and were indeed undetectable within photoreceptor apoptotic processes when different inhibitors were applied (Perche et al., 2008). Two other main factors in the apoptotic process, Bax and c-Jun, were found to be increased at P21d and P28d, whereas Bcl-2 was unchanged, both chronologically and spatially (Katai et al., 2006). AIF nuclear translocation was also found in the RCS rats' apoptotic photoreceptors. When translocation was inhibited, photoreceptor apoptosis was prevented, leaving the upregulated expression of Bcl-2 to control mitochondrial membrane integrity and the release of AIF (Murakami et al., 2008). During retinal development, mitochondrial RNA damage (mtDNA deletion) has also been found to be closely associated with photoreceptor death induced by hypoxia and to have a longer duration and more serious deletion rate compared to SD rats (Bravo-Nuevo et al., 2007).

Through the application of inhibitors of the caspase pathway, caspase-3 or -7 and caspase-1 or -4 were found

to be involved in the cell death mechanism at the early stages of apoptosis, with caspase-3 dominating the apoptotic process and acting as a downstream factor of caspase-145 (Perche et al., 2008). The *p53* gene was also found to be related to the regulation of the photoreceptor apoptosis by increasing its expression significantly at the early stages of photoreceptor apoptosis (Liu and Zhu, 2000).

Autoimmune responses have also been suggested to participate in photoreceptor apoptosis. For instance, antigenic material has been suggested as being released from dead photoreceptor cells and exposed to serum autoantibodies, destroying the blood-retinal barrier and attracting activated pro-inflammatory cytokines/chemokines, which in turn produce microglia/microphages. Each of these actions augments photoreceptor apoptosis (Kyger et al., 2013).

Increased pro-microglia-derived nerve growth factor protein has been observed in RCS rats to be released by activated retinal microglia. This process may promote the degeneration of photoreceptor cells via the p75 neurotrophin receptor, which is a member of the tumor necrosis factor receptor superfamily and contains a 'death domain' motif that has been found to be expressed in the dystrophic retina IS/OS and PRE (Sheedlo et al., 2002; Srinivasan et al., 2004).

Cells are particularly vulnerable to oxidative stress when iron has accumulated in the outer retina, which might be a result of the impaired RPE-photoreceptor interaction, which blocks the outer retina's iron delivery pathway (Yefimova et al., 2002).

During this progression, ER stress levels have been shown to be high, ultimately leading to cell death and retinal dystrophies when the functions of the retina have been disrupted by misfolded proteins (Tabas and Ron, 2011). The mRNA of binding of immunoglobulin protein (BiP), which can be transcriptionally induced by the ER during stress, has been shown to be increased during the later phases of RCS retinal degeneration, suggesting that the ER stress is not the initiator but, rather, an amplifier of retinal damage (Kroeger et al., 2012).

Megalin acts as a transfer vector and has been shown to be expressed in the region of IS/OS and nerve fiber layers in the normal retina. However, this protein is lost with age in RCS retinas, leading to the impairment of metallothionein-I+II transportation (from, e.g., Müller cells) into degenerating photoreceptors, potentially limiting its role protecting the retina cells against oxidative damage and regulating the survival and regeneration of RGC axons (Wunderlich et al., 2010).

Rods and cones have been found to regulate melanopsin mRNA expression. The rhythmic expression of melanopsin remains unchanged before the degeneration of the photoreceptors, but the pattern is greatly reduced and not rhythmic at P60d (Sakamoto et al., 2004). Melanopsin is also thought to be regulated by dopamine, which participates in the formation of connections between amacrine cells and mRGCs (Armata et al., 2006; Vugler et al., 2007).

Müller cells have been shown to become activated when the expression of ERK and GFAP has increased dramatically with retinal degeneration. This process is thought to be a secondary response to retinal degeneration, as ERK and GFAP expressions increased significantly when Müller cells from a normal rat were co-cultured with RCS retinal cells (Zhao et al., 2010).

Retinal neurotransmitters

Dopamine, glutamate, aspartate, ACh and GABA comprise the neurotransmitters of the retinal pathways, with DA, glutamate and γ-aminobutyric acid (GABA) considered to be the major players. Photoreceptors and bipolar and ganglion cells use glutamate, whereas horizontal and most amacrine cells in the parallel pathway use GABA as their neurotransmitters (Barnstable, 1993). Compared to normal rats, most of the retinal neurotransmitters in RCS rats were not significantly changed at 4 weeks of age, except for a decrease in taurine reaching severe levels by the later stages. At nearly 6 months of age, the amounts of retinal glutamate, DA and aspartate are significantly decreased, whereas the levels of ACh and GABA are unaffected. Glycine expression was found to be significantly higher than in control rats (Okada et al., 2000).

The secretion of endogenous dopamine and its metabolites has been shown to be impaired in the dystrophic retina as early as 3 weeks of age (Hankins and Ikeda, 1994) in a process almost entirely dependent on photoreceptors (Frucht et al., 1982). This altered rhythmicity has been shown to have farther-reaching effects than the observed reduced amplitude in the RCS retina (Doyle et al., 2002). A microRNA, mir133b, has been shown to suppress dopaminergic amacrine function via its overexpression in the early stages of degeneration, downregulating the transcription factor Pitx3 of dopaminergic amacrine cells (Armata et al., 2006; Li et al., 2012b).

Glutamate metabolism in the degenerating RCS rat retina is impaired, and excitotoxic glutamate has been shown to contribute to retinal cell loss. Before P60d, the increasing ratio of VGLUT-1 (glutamate binding protein in retinal neurons) to PKCα that exists mainly in bipolar cells indicates that the percentage of living rod bipolar cells has increased to become the main glutamate-releasing and storage neurons in the retina during retinal degeneration. The subsequent decrease at P90d suggests a reduction in glutamate release from bipolar cells to ganglion cells as well as the deafferentation of ganglion cells. The expression of GLAST, which binds free glutamate in the extracellular space, has been shown to be unchanged throughout retinal degeneration in RCS rats, in contrast to the results of rd1 mice (Delyfer et al., 2005; Liu et al., 2013).

The glutamate binding sites in the retina include N-methyl-d-aspartate (NMDA), ionotropic, amino-methyl-

propionic-acid (AMPA), metabotropic glutamate and kainate glutamate receptors (Grunder et al., 2001). Neurotransmission from these photoreceptors to rod bipolar and ON-cone cells has been found to be mediated by the metabotropic glutamate receptor mGluR6, whereas that from photoreceptors to OFF-cone bipolar cells is mediated by kainate ionotropic glutamate receptors (DeVries and Schwartz, 1999). In IPL, the kainate glutamate receptors have been shown to be expressed at a higher level in the retinas of RCS compared to normal mice on P35d (Stasi et al., 2003). The expression of the subunits NRI and NR2B of the glutamate receptor NMDA has been shown to be reduced after P40d; however, the strong expression of NRI in the Müller cell processes located in the inner retina has been shown to occur earlier than the photoreceptor degeneration (Grunder et al., 2001). The expression levels of the mGluR6 mRNA decrease during the early stages but increase during the later stages of degeneration in RCS (Armata et al., 2006). These changes have been suggested to be related to the loss of electrical activity in the retina as a consequence of photoreceptor degeneration. The undeclined NMDA receptors in the RCS rats, which are associated with retinocollicular transmission, suggest the neural plasticity of the degenerated retinas (Pothecary et al., 2005).

Expression of other factors

Some factors are regulated or re-expressed when degeneration occurs and regeneration is activated. From early postnatal life onward, the expression of nerve growth factor and brain-derived neurotrophic factor in the degenerated retina and in the geniculate nucleus during late-stage degeneration is significantly affected compared to control eyes (Amendola et al., 2003). The significantly decreased Rhodopsin kinase (RK) expression in RCS rats, both in the retina and in the pineal gland, suggests the abnormal regulation of rhodopsin phosphorylation. Arrestin expression in the RCS showed no significant difference compared to control retinas or PGs, whereas no recoverin or other G-protein coupled receptor kinase expression was found in RCS PG during postnatal weeks 3-5 (Takano et al., 2003). Nitric oxide (NO) expression is also unchanged in RCS rat retinas and has been reported to be both neurodestructive and neuroprotective in the central nervous system (Sharma et al., 2001).

Factors with retinal protective potential have been reported to be increased. The crystallins, which belong to the apoptosis-suppressing small HSP subfamily, are expressed in the retina and brain. The cytoprotective effects of crystallins might be activated during the initial stage of degeneration. The retinal contents of all crystallins increase with photoreceptor loss compared to the age-matched normal controls, and more acidic crystallins can be detected at P30d. Still, at P40d, the contents begin to reduce, resembling those of normal retinas by P60d (Organisciak et al., 2006). Nestin is expressed in some stem or progenitor cells during retinal development and re-expressed in response to acute damage in adult retinas, a process thought to originate in activated Müller cells. In dystrophic RCS rats, nestin protein levels decrease from P0d to P30d but increase progressively when photoreceptor degeneration begins. However, in control rats, the nestin levels decrease from P7d to P20d and remain low from then on. Dysregulation of the stabilization and/or degradation processes of this protein has been suggested, as the mRNA-level changes have not been shown to synchronize with the protein (Valamanesh et al., 2013).

Blood vessel remodeling

Changes in the blood vessels are characterized by neovascularization and non-neovascular remodeling. Neovascularizations have been observed after low perfusion, especially in the middle and peripheral portions of the retina. Growth-associated factors and their receptors, including Ang-2, somatostatin, VEGF and the VEGF receptors, are significantly upregulated up to P29d in RCS rats (Prokosch et al., 2011). Apelin, but not VEGF-related neovascularization, is likely to be a major contributor in the remodeling of vessels in RCS rats. When the differentially expressed genes in retinal microvessels were checked, Apelin and its receptor Aplnr were found to be present at increased levels; when these genes were suppressed, vascular remodeling was altered (McKenzie et al., 2012).

Conclusions

RCS rats play an important role in the exploration of RP, and a considerable number of studies have revealed changes and mechanisms consistent with retinal degeneration in these rats. These morphologic and pathophysiologic retinal changes have been documented as the markers of the cells are investigated, providing useful information on the primary and secondary cellular alterations in this disease. Dysfunctional cell phagocytosis and cell death initiate the degenerative process, and then the metabolism and microenvironment abnormally accelerate the process. As components of the neural system, the transmitters and other neural factors are also impaired. Although we have made advances in mechanistic studies with treatment-guiding potential, areas of debate remain, and unforeseen complications, e.g., autophagic cell death and neurotransmission, may yet emerge.

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