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Chapter 7

PTEN INVOLVEMENT IN EYE EMBRYOLOGY AND PATHOLOGY

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ABSTRACT

The tumor suppressor gene *PTEN* is a plasma-membrane lipid phosphatase, which negatively regulates the PI3K/Akt cell survival pathway. The crucial role of PTEN in several cellular processes, as well as the different mechanisms by which it exerts its functions, could explain its involvement in the pathogenesis of numerous diseases, including a wide variety of tumors and a group of rare autosomal dominant syndromes, known as the *PTEN* Hamartoma Tumor Syndrome (PHTS).

This chapter summarizes the current knowledge about the implications of PTEN in eye development, degenerative ocular diseases, as well as ocular tumors. Growing evidences, using different model systems, such as *Drosophila*, zebrafish, and transgenic mice models, are supporting essential functions of PTEN during eye formation, by affecting cell size, proliferation, and migration in a cell-type specific manner. In particular, suppression of the PI3K/Akt signaling pathway by PTEN seems to play an important role for proper retinal neurogenesis, and to define a functional retinal architecture.

We also discuss how dysregulation of PTEN expression could be responsible for the pathogenesis of retinal degenerations, such as retinitis pigmentosa and age-related macular degeneration, and how germline mutations associated with PHTS could provide a clinical spectrum which encompasses several syndromes with variable ocular involvement. We finally evaluate the contribution of PTEN in the biology of ocular tumors.

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INTRODUCTION

For several years complete or partial deletions of human chromosome 10 had been frequently observed in different malignant tumors. In 1997, homozygous mutations at the 10q23 locus in brain, breast, kidney, and prostate cancers led to the identification of a novel candidate tumor suppressor gene, designated *PTEN/MMAC1* gene [1, 2]. At the same time, germline *PTEN* mutations were also identified in patients affected by Cowden disease, Bannayan-Zonana syndrome, and Lhermitte-Duclose disease, three autosomal dominant syndromes, whose common feature is the development of multiple benign tumors [3, 4]. The observation that PTEN could drive tumorigenesis in different tissues and cell types suggested that its function was crucial in several cellular processes.

The PTEN/MMAC1 protein contains the signature motif of the catalytic domain of protein tyrosine phosphatases [1, 2]. *In vitro* and *in vivo* studies demonstrated that PTEN functions as a plasma-membrane lipid phosphatase, which dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) in order to generate phosphatidylinositol (4,5)-bisphosphate (PIP_2) [5], thereby negatively regulating the phosphoinositide 3-kinase (PI3K) /Akt pathway, which is involved in cell growth and survival [6]. It is not surprising therefore that *PTEN* mutations are preferentially located in its phosphatase catalytic domain [7].

In mice, homozygous *Pten* mutations are embryonic lethal, while heterozygous mutations predispose to the development of tumors, partially resembling the spectrum of cancers observed in patients with Cowden syndrome [8-10]. In a high percentage of cases, epithelial and nervous tumors developed in *Pten*+/- mice exhibit loss of heterozygosity at the *Pten* locus, indicating the importance for loss of PTEN function in tumor formation and progression [11-13]. However, in humans, tumors arising in Cowden's syndrome patients do not always show loss of the wild-type *PTEN* allele [14].

Interestingly, the extent of PTEN inactivation seems to be the key determinant in cancer predisposition and progression in a tissue-specific manner, as it has been shown by generating hypomorphic *Pten* mutant mice [15, 16]. In some instances, the complete loss of PTEN may be actually less tumorigenic than a partial loss, because total depletion of PTEN expression can drive a p53-dependent cellular senescence pathway, which in turn can slow down tumor formation [17]. Thus, PTEN defines a new paradigm of haploinsufficiency, defined "obligate haploinsufficiency", where the maximum of the tumorigenic potential of a cell is achieved with an intermediate PTEN expression level [17].

Regulation of PTEN expression and activity happens not only at the genetic level, but also by transcriptional and post-transcriptional mechanisms, such as epigenetic silencing [18], miRNAs regulation [19], post-translational modifications [20], and aberrant protein localization [21]. Furthermore, several PTEN interactors can positively or negatively affect PTEN function, by modifying its conformation, stability and cellular distribution [22].

Most of our current knowledge of PTEN function derives from cancer-related studies, but several data also support its involvement in the pathogenesis of other diseases than cancer.

Brain and liver represent tissues where PTEN dysregulation leads to a broad spectrum of diseases. In the brain, PTEN mutations are best known in the pathogenesis of glioblastoma, the most common and aggressive malignant primary brain tumor in humans; PTEN seems also to play a role in central nervous system pathophysiology [23, 24], being involved in neuronal injury [25], and in neurological and psychiatric disorders, such as Alzheimer [26, 27] and Parkinson [28]. In the liver, dysregulation of PTEN activity affects hepatic insulin sensitivity, and triggers the development of non-alcoholic fatty liver diseases (NAFLD) [29]. HBV and HCV infections can affect PTEN expression levels, as well as alcohol-related injury [30]. *PTEN* point mutations/deletions are moreover associated with liver malignancies, such as hepatocellular adenomas and carcinomas [31, 32].

Recent reports are shedding light on the importance of PTEN expression/activity in the eye, not only as an essential protein during eye development, but also as a key regulator in degenerative ocular diseases, as well as ocular tumors.

Role of PTEN during Eye Development

During the last years PTEN has emerged as a novel regulator of the formation and maintenance of the retina.

In the adult retina, there are six major types of differentiated neurons (rod and cone photoreceptors, bipolar, amacrine, horizontal, and ganglion cells) and one type of glial cells (the Müller glia) that are organized into three distinct cellular layers. The differentiated retinal cell types originate from a pool of multipotent retinal progenitor cells (RPCs) during a defined temporal window [33]: in mice, ganglion cells are generated first, followed by cone photoreceptors, horizontal cells, and most of the amacrine neurons; all the other retinal cell types are mainly produced postnatally [34]. Differentiation of RPCs into specific retinal cell types happens under the stimuli of genetic signals and/or environmental changes [33, 35]. Besides the complex network that could affect RPCs fate, these cells can only maintain the characteristics of progenitor cells or differentiate into specific neuronal cell types.

In the outer retina, photoreceptor cells are in contact with horizontal and bipolar cells within the outer plexiform layer (OPL), while in the inner retina ganglion cells are connected through synapses to the amacrine and bipolar interneurons in the inner plexiform layer (IPL).

In P0 mice retina, PTEN is expressed predominantly in the inner retina (corresponding to most-mitotic retinal ganglion cells and amacrine cells), while weaker expression is observed in the ventricular zone, where there are postnatal retinal progenitors and prenatally produced photoreceptors. In the mature retina, PTEN is homogeneously distributed in the inner plexiform layer and in the ganglion cell layer, and low expressed in the inner nuclear layer, where bipolar, horizontal and amacrine cells are located [36, 37].

Suppression of PTEN expression in mice RPCs causes severe morphological retinal defects, suggesting that PTEN function is crucial for normal retinal development [36]. This observation is in agreement with the severely disrupted retina morphogenesis observed by overexpressing PI3K in the mouse retina [38]. PTEN seems to affect retinal development in a cell type-specific manner: in *PTEN-cKO* retinas, the number of ganglion cells is significantly reduced, the distribution of amacrine cells is severely affected, rod photoreceptors present shortened inner/outer segments and are dramatically decreased in number, and Müller

gliogenesis is enhanced [36, 37]. The aberrant distribution of amacrine cells within the inner plexiform layer has been attributed to the continuous growth of their cell bodies and processes [37].

The importance of the PI3K/Akt signaling pathway in the differentiation of RPCs is even supported by the observation that this pathway is more active in post-mitotic retinal neurons than in RPCs [39]. Neurogenesis in RPCs lacking PTEN is faster than the normal developmental timing, resulting in a premature depletion of RPCs from the mature retina. This could be partially explained by a reduced Notch intracellular signaling activity due to Akt hyperactivation, as overexpression of the Notch1 intracellular domain (NICD) could restore the developmental retinal defects observed in *PTEN-cKO* mice [39].

The three-dimensional architecture of the retina and the precise distribution of the retinal neurons are essential for the complete sampling of the visual field. Retinal neurons cell bodies are distributed in a given position within a nuclear layer, which is peculiar for any given cell type, and they maintain a certain distance between each other [40]. Arborisations of retinal neurons are also strictly organized to create a sort of cellular array, which is able to provide the proper flow of signals from the retina to the brain [41]. The role of PTEN as a regulator of terminal arborisation *in vivo* has been shown in *Xenopus* [42]. In mice, Pten regulates the positioning and neurite arborisation patterns of a subset of retinal cells (ganglion, amacrine and horizontal cells), therefore contributing to the establishment of a correct retinal architecture [37].

Drosophila represents an alternative model organism that can help to gain insight into the function of PTEN during eye development, since expressing either human or fly PTEN in *Drosophila* shows similar eye phenotype [43]. *Drosophila* has a homolog of human *PTEN* gene, designed *dPTEN*. The two genes are similar in length and share sequence similarities in both the N-terminal catalytic domain and the C-terminal region. Homozygous *dPTEN* mutations are embryonic lethal, suggesting that *dPTEN* encodes a vital function needed for early *Drosophila* development; if dPTEN is lost during eye development, mutant ocular cells have an enlarged size, but the composition and orientation of photoreceptor cells is preserved [43-45]. The increase in *Drosophila* eye size in *dPTEN* mutants is the result of an increase in cell number, but also in the average size of each mutant cell. As expected, after overexpression of dPTEN in the eye disc, a dramatic reduction of eye size is observed, due to cell death in differentiating cells, but not in the proliferating ones [43]. Reversal of this latter phenotype can be achieved by overexpressing in *Drosophila* the human thioredoxin-1 (hTrx-1) protein, which binds to the C2 domain of PTEN, inhibiting its phosphatase activity [46].

Zebrafish has also two orthologs of the mammalian *PTEN* gene, designed *ptena* and *ptenb*, whose expression is predominantly found in the eye, in the central nervous system, in the branchial arches, and in the pectoral fins [47]. Knocking down either of the two genes in zebrafish embryos causes an increase of phosphorylated Akt, indicating that each of them possess the same phosphatase activity as in mammals [47]. During zebrafish embryonic development, ptena and ptenb have redundant functions, because neither of the two single homozygous mutants (*ptena-/-* or *ptenb-/-*) shows any embryonic phenotype. Surprisingly, *ptenb-/-* zebrafish develops ocular neural tumors later in life, despite the redundancy of ptena expression in the adult eye [48]. These observations suggest that the two genes have most likely redundant activities during zebrafish development, but later in life acquire specific functions, as it could be the case for ptenb in the eye.

PTEN Involvement in Retinal Degenerative Diseases

Photoreceptor cells (rods and cones) are specialized retinal neurons that are capable of phototransduction; cone photoreceptors mediate color vision, while rods, being more sensitive to light, are responsible for vision in dark conditions. In the human retina, there are approximately 5 million cones and 120 million rods; the highest concentration of cones is found in the macula, the central part of the retina, which provides visual acuity, while rods are mainly distributed in the periphery of the retina.

Photoreceptor degeneration (PD) is the major cause of blindness in industrialized countries, and represents an extremely heterogeneous group of genetic diseases. Since many genes and environmental factors are known to be responsible for photoreceptor degeneration, it has been suggested that PD can be considered as a complex trait [49]. Inherited forms of PD are mainly monogenic diseases; retinitis pigmentosa (RP) is one of the most common types, having a prevalence of 1 in 4,000 in the worldwide population. It can occur alone or in combination with other clinical manifestations as syndromic RP. Patients affected by the disease complain about a progressive loss of vision in the dark (-nyctalopia- due to rods degeneration) in their early or middle life; their peripheral vision becomes more and more constricted resulting in the so-called tunnel vision, and finally, after cones degenerate as well, the situation deteriorates to loss of the central vision.

The *rd* mutant mouse has been extensively used as a model for human retinitis pigmentosa, since it mimics an autosomal recessive form of RP, due to mutations in the rod-specific *cGMP-phosphodiesterase β subunit* (*PDE β*) gene [50, 51]. In this model, rods start to degenerate at about postnatal day 8, but even before the onset of rods degeneration, an increase of cGMP molecules is observed in the retina, due to the dysfunction in cGMP phosphodiesterase activity [52]. In *rd* mice, degeneration of photoreceptor cells is therefore related to a metabolic imbalance caused by elevated levels of cGMP in the retina.

Caspase-dependent and -independent apoptosis has been shown to be the main mechanism of photoreceptor cell death in animal models of inherited retinal degenerations [53], including the *rd* mouse strain [54]. In *rd* mice retinas, cytocrome c is released from the mitochondria and the pro-apoptotic protein BID and its upstream regulators caspase-8 and p38 mitogen-activated protein kinase (MAPK) are activated during photoreceptor degeneration [55]. PTEN is known to promote death-receptor mediated apoptosis through a FADD (Fas associated death domain) -dependent pathway, leading to activation of caspase-8, which in turn cleaves BID [56]. In *rd* mice retinas, PTEN expression is higher than in the WT retinas at postnatal day 13 and 14, corresponding to the peak of photoreceptor degeneration [57]. Up-regulation of PTEN activity in *rd*-mice retinas is probably due to a weaker expression of its negative regulator Src-p60, belonging to the family of Src protein-tyrosine kinases [57, 58]. Inhibition of Akt survival pathway by PTEN is therefore responsible for photoreceptor cell death in the *rd* mouse model, and the subsequent Akt activation after most photoreceptor cells have died is most likely a necessary event for the survival of the remaining retinal cells [57].

The retinal pigment epithelium (RPE) is a layer composed of neuroepithelial cells that are located between the photoreceptor cells and the blood vessels of the choroid layer. RPE cells

protect the photoreceptors from cell death, either by uptaking their metabolic wastes and by providing them nutrients. Reactive oxygen species (ROS) are accumulated in the retina during the process of phototransduction or after exogenous insults, and can led to retinal degeneration if not promptly eliminated by the photoreceptor antioxidation machinery and by RPE cells. In old RPE cells, the homeostatic antioxidative mechanisms can fail to function because of excessive exposure to oxidative stress during their life. When RPE cells start to degenerate a concomitant loss of photoreceptor cells is observed, and degeneration of both cell types is a primary cause of age-related macular degeneration (AMD). AMD represents the major cause of visual impairment and blindness in older adults (>50 years), resulting in loss of vision in the central part of the retina (the macula). Impairment of the structural integrity of the RPE leads to retinal degenerative diseases such as AMD and some forms of retinitis pigmentosa. RPE-specific *Pten* deletions in mice cause RPE cells degeneration and migration out of the retina, death of the photoreceptor cells, macrophages invasion of the retina, and retinal neovascularization [59]. All these phenotypes resemble the pathology of AMD in humans. Interestingly, these events have not only been observed by genetic depletion of *Pten* in RPE cells, but also by inactivation of Pten function due to oxidative stress. ROS can in fact phosphorylate PTEN at specific sites (S380/T382/T383), reducing its enzymatic activity [59, 60], and leading to the consequent accumulation of phosphatidylinositol (3,4,5)-trisphosphate. The PI3K/Akt pathway enters therefore a positive feedback loop, promoting the continuous generation of ROS and the development of AMD [61]. Inactivation of Pten in RPE cells seems to be a common feature of AMD mouse models, where ROS levels are increased [60]. Once the balance of PI3K/PTEN signalling events is perturbed, it affects the maintenance of RPE junctions, leading to the disruption of the normal RPE architecture [61].

Ocular Manifestations of *PTEN* Hamartoma Tumor Syndrome

The *PTEN* hamartoma tumor syndrome (PHTS) is a group of autosomal dominant disorders caused by *PTEN* germline mutations, whose common clinical features encompass multiple benign malformations that resemble a neoplasm in the tissue of their origin (hamartomas), and the predisposition to develop malignancies. It includes four syndromes classified as Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome that present different phenotypic manifestations. The only criterion for the classification of PHTS is therefore based on the presence of a germline *PTEN* mutation.

CS usually manifests by the age of 20 years with macrocephaly, mucocutaneous lesions, thyroid dysfunctions, and an elevated risk to develop breast, thyroid, and endometrial carcinomas [62]. BRRS is characterized by macrocephaly, intestinal polyposis, lipomas, and pigmented macules of the glans penis. PS is a highly variable disorder, which causes overgrowth of multiple tissues, leading to the development of skin and bones tumors. Proteus-like syndrome refers to individuals with significant clinical features of PS, but who do not meet the diagnostic criteria for PS. Variable ocular features have been reported among the clinical manifestations of PHTS (Table 1).

Table 1. Ocular manifestations of PHTS

	Ocular manifestation	Reference
Cowden syndrome	Retinal glioma Retinal and optic nerve drunsen	Nuss at al. 1978
	Cataract Angioid streaks Vascular anomalies	Starink et al. 1986
	Choroidal hamartoma Conjunctival papilloma	Wells et al. 1994
	Congenital nystagmus	Yang et al. 1994
	Bilateral glaucoma Micro-ophthalmia Bilateral posterior uveitis Cataract	Vantomme et al. 2001
	Retinal angiomatous lesions	Gicquel et al. 2003
	Proliferative retinopathy	Mansoor et al. 2012
Bannayan-Riley-Ruvalcaba syndrome	Pseudopapilledema	Dvir et al. 1988
Proteus syndrome	Strabismus Nystagmus High myopia Retinal pigmentary abnormalities	De Becker et al. 2000
	Epibulbar cystic lesions Congenital abnormalities of the retina Congenital lack of vessels in the uvea Calcified drusen in the optic disc Optic atrophy Hyperpigmentation of the iris Retinal pigmentary changes	Gilbert-Barness et al. 2000
	Myopia Mild calcific band keratopathy Cataract Abnormal vitreous structure Vitreous haemorrhage Serous retinal detachment Chorioretinal hamartoma	Sheard et al. 2002
	Myopia Conjunctival capillary haemangioma Coloboma of the optic disc	Venugopalan et al. 2001
	Nystagmus Retinal dysgenesia Retinal pigmentary abnormalities Optic nerve hypoplasia High myopia Strabismus	Sanchez-Lopez et al. 2007

In Cowden syndrome, the most common ocular abnormalities are cataract, angioid streaks, and vascular anomalies [63]. Retinal glioma [64], choroidal hamartoma [65], and conjunctival papilloma [65] have been observed in few patients, among which some presented Lhermitte-Duclos disease features [65]. Other ocular phenotypes include glaucoma, micro-ophthalmia, and uveitis [66]; retinal vascular abnormalities reported to date are

angiomatous lesions [67] and proliferative retinopathy [68]. In a single case report congenital nystagmus has been observed as a new feature of this syndrome [69].

In Bannayan-Riley-Ruvalcaba syndrome, the only ocular manifestation that has been reported is pseudopapilledema [70].

In Proteus syndrome, strabismus and epibulbar tumors are the most frequently occurring ocular abnormalities [71]. Myopia, keratopathy, cataract, nystagmus, abnormal vitreous structure and vitreous hemorrhage, retinal serous detachment, chorioretinal hamartoma, as well as congenital abnormalities of the retina, lack of choroidal vessels, and optic atrophy have been also observed as single case reports [72, 73, 74-76]. Unfortunately patients with PS do not systematically undergo ophthalmologic examination, therefore the incidence of ocular manifestations in PS is unknown [74, 75].

Interestingly, a rare tumor originating from Schwann cells (schwannoma) that are the principal glia of the peripheral nervous system, developed for the first time in the choroid of a patient with PHTS. At the molecular level the tumor showed a unique combination of reduced PTEN expression and absence of the protein Merlin, encoded by the *NF2* gene, which is a cause of both sporadic and familial schwannomas [77].

PTEN in the Biology of Ocular Tumors

PTEN dysregulation has mainly been decribed in melanoma arising in the richly vascularized uvea [78], but also in skin melanoma [79-81] and even in very rare melanoma arising in the conjunctiva [82]. Furthermore, some lines of evidence also suggest an involvement of PTEN deregulation in the pathogenenesis of epithelial cutaneous tumors such as squamous cell carcinoma [83-86]. To the best of our knowledge, specific dysregulations of PTEN in cutaneous melanoma and squamous cell carcinoma of the eyelids have not been described yet.

Uveal melanoma is not only the most common ocular melanoma (85%), but it is also the most common primary intraocular tumor of the adult. Contrary to skin melanoma whose incidence is rising and where UV exposure has been shown to be mutagenic [87], the incidence of uveal melanoma (4.3-10 per million per year) has not increased over the past years [88]. This tumor more frequently affects whites, peaking in females at the age of 60 years and males at the age of 70 years. The mortality of uveal melanoma due to metastatic disease occuring in 50% of the patients has not decreased over the past year and effective therapy are still lacking [89].

Compared to other solid tumors, ocular melanoma presents rather homogeneous chromosomal rearrangements [90], that accumulate in a non-random temporal sequence [91]. Mutations of *GNAQ*, *GNA11* and *BAP1* are major contributors in the development of uveal melanoma. *GNAQ* and *GNA11* are heterotrimeric G-protein alpha subunits involved in signal transmission between G-protein coupled receptors and downstream effector [92]. *GNAQ* and *GNA11* activating mutations in exon 5 of the GTPAse domain seem to appear early in the development of uveal melanoma. These somatic mutations are found in 83 % of uveal melanoma [93]. *BAP1* mutations on chromosome 3 seem to occur later in tumor progression, usually in tumors that have already lost a copy of chromosome 3 and are found in metastatic uveal melanoma [94]. BAP1 functions as a deubiquitinating enzyme that binds to BRCA1 and BARD1 and acts as a tumor suppressor gene [94].

Although no cytogenetic alterations at the 10q23 locus nor *PTEN* mutations have been identified in uveal melanoma cell lines [95], loss of large portions of chromosome 10 have been reported in about 30% of primary uveal melanomas [96]. Decreased or complete loss of PTEN expression in a series of primary uveal melanomas is associated in about 40% of the samples with loss of heterozygosity at the *PTEN* locus for at least two polymorphic markers [78]. Interestingly, in uveal melanomas, as well as in many other primary tumors, complete PTEN loss has an unfavourable prognostic significance [78]. In these tumors, an increased rate of aneuploidy is strongly associated with PTEN downregulation, suggesting that loss of PTEN favors genomic instability [91]. If PTEN expression is completely abolished in uveal melanoma, patients have a median survival rate of 60 months compared with more than 120 months for patients with normal PTEN expression [89]. LY294002 is a potent pharmacological inhibitor of PI3K activity, which is able to decrease cell proliferation in uveal melanoma cell lines, but unfortunately due to its poor solubility and high toxicity, this molecule cannot be used as anticancer drug for patients [97]. Nevertheless, other PI3K inhibitors such as XL147 are under clinical investigation, and look more tolerable than LY294002 [89]. Inhibition of the PI3K/Akt pathway can be also achieved using inhibitors of Akt such as perifosine, GSK2141795, GSK690693, and MK2206 that are currently used in clinical trials [89].

The role of PTEN as a tumor suppressor gene in the development of cutaneous melanoma has been extensively investigated, notably in cell lines [81, 98, 99, 100]. *PTEN* mutations were more commonly found in cultured melanoma (27%) than in primary tumors (7%) and metastatic tumors (15%) [101]. In a recent study [102], *PTEN* promoter methylation was found in 60% of the cases of cutaneous melanoma (120/200) and was independently associated with an increased risk of death. Activating mutations in the kinase domain of *BRAF* (V600E) are the most common in skin melanoma and lead to activation of the MAP kinase pathway [103]. In a mouse model, the presence of *BRAF* mutation (V600E) in melanocytes led to development of melanoma that was also associated with metastasis, only if combined with concomitant PTEN gene silencing [104]. Moreover, some evidence also suggest that the resistance to BRAF inhibitor PLX04032 might be partially associated with the presence of simultaneous PTEN loss [81,105,106].

In conjunctival melanoma PTEN nuclear expression was found to be significantly reduced comparing to benign nevi, leading to up-regulation of the anti-apoptotic protein Bcl-2 [82].

H-RAS mutations, found in 22% of cutaneous human squamous cell carcinoma [107] can lead to activation of the RAF/MEK/ERK pathway and phosphatidylinositol 3-kinase (PI3K/AKT/mTOR) pathway. The activation of these pathways has been involved in keratinocytes proliferation and squamous cell carcinoma formation in mice models [108]. Downregulation of *PTEN* in a mice model led to hyperproliferation of keratinocytes, hyperkeratosis and development of spontaneous squamous cell carcinoma [83]. Further evidence suggested that *H-RAS* activation and complete *PTEN* loss were mutually exclusive [109]. More recently, PTEN was shown to be significantly reduced at the protein level in human actinic keratosis and squamous cell carcinoma comparing to normal skin [110]. In this study, downregulation of *PTEN* in mice predisposed to squamous carcinoma formation upon UVB exposure through downregulation of the protein xeroderma pigmentosum involved in nucleotide excision repair. In 2011, deletion of the developmental factor *Grainy headlike 3* (*GRHL3*) in mice keratinocytes predisposed to squamous cell carcinoma formation upon

exposure to chemical carcinogens [85]. *PTEN* was identified in this study as the transcriptional target of *GRHL3*. *Grhl3*$^{+/-}$*/Pten* $^{+/-}$ mice developed aggressive squamous cell carcinoma, a phenotype that could almost completely be prevented if PTEN expression was restored. This study furthermore demonstrated that *GRHL3* and *PTEN* expression were downregulated upon miR-21 upregulation in squamous cell carcinoma cell lines, providing a potential explanation for the low level of PTEN expression in squamous carcinoma in the absence of genetic alterations or epigenetic silencing of *PTEN*.

CONCLUSION

Since discovery, PTEN has been shown to be involved in a wide range of cellular processes affecting the development and homeostasis of several human tissues.

This chapter aimed to provide an overview of the role of PTEN during eye development and in ocular diseases. Recent literature from different model organisms shed light on the crucial roles of PTEN in various aspects of eye development and diseases. During ocular development PTEN has been shown to be an essential modulator of cell growth, differentiation, migration, and arborisation, in a cell-type specific manner. It is therefore not surprising that dysregulation of PTEN expression in the eye can lead to degenerative ocular diseases, such as retinitis pigmentosa and age-related macular degeneration, and that germline *PTEN* mutations may predispose to the development of ocular abnormalities. In the context of *PTEN* hamartoma tumor syndrome ophthalmologic examination should be therefore systematically performed, in order to define the incidence and assess the risk for the patients to develop ocular diseases that would otherwise lead to visual impairement.

As PTEN downregulation occurs in uveal melanoma, anti-cancer drugs targeting the PI3K/PTEN/Akt/mTOR pathway are at the moment under investigations in clinical trials, but more research is definitely needed to improve patient outcomes.

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