Physiologic Properties of Müller Cells from Human Eyes Affected with Uveal Melanoma

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Purpose. To study physiologic characteristics of human Müller cells from healthy and pathologically altered eyes.

Methods. Human tissue was used from organ donors and from patients affected with uveal melanoma. Several melanoma eyes also showed retinal detachment. Incubation of freshly prepared slices with a commercial vital dye preferentially stained Müller cells. The Müller cell response to hypotonic stress was observed by recording the cross-sectional area of cell somata. Electrophysiologic properties were investigated in parallel in whole-cell patch-clamp experiments.

Results. Inward K^+ currents mediated by inwardly rectifying Kir channels were significantly decreased in Müller cells from eyes with uveal melanoma compared with healthy controls. This was accompanied by a decrease of the membrane potential. Both effects were stronger in cells from eyes where the melanoma had caused a widespread retinal detachment. Application of a hypotonic solution did not affect Müller cells from healthy organ donors. By contrast, Müller cells from some melanoma eyes increased their soma size in response to hypotonic solution. This effect was aggravated in cells from eyes with widespread retinal detachment. The inflammatory mediator, arachidonic acid, could induce Müller cell swelling, whereas anti-inflammatory substances reduced the swelling.

Conclusions. The experiments with human tissue confirm earlier data from animal models for retinal pathologies about typical alterations of reactive Müller cells. Hypotonic stress induced Müller cell swelling preferentially in cells from melanoma-affected eyes that displayed decreased inward current amplitudes. Widespread melanoma-associated retinal detachment potentiated the pathologic alterations of Müller cells. (*Invest Ophthalmol Vis Sci.* 2012;53:4170–4176) DOI: 10.1167/iovs.12-9746

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Supported in part by Deutsche Forschungsgemeinschaft Grants PA 615/2-1 (TP) and GRK 1097/2 and RE 849/12 (AR) and the German Federal Ministry of Education and Research Grant PtJ-Bio/0315883 (MF and AK).

Submitted for publication February 23, 2012; revised May 11 and May 21, 2012; accepted May 26, 2012.

Disclosure: **A. Grosche**, None; **T. Pannicke**, None; **A. Karl**, None; **I. Iandiev**, None; **M. Francke**, None; **P. Wiedemann**, None; **A. Reichenbach**, None; **A. Bringmann**, None

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Müller cells, the principal macroglial cells of the retina, are involved in a variety of retinal diseases and injuries.[1,2] These cells then undergo a process of reactive gliosis that is characterized by certain alterations in morphology, physiology, and gene expression.[1,2] Basically, reactive Müller cells become hypertrophic and increase the expression of intermediate filaments, such as glial fibrillary acidic protein (GFAP).[3,4] Moreover, they display reduced inward currents (mediated by inwardly rectifying K^+ [Kir4.1] channels) in many but not in all cases of retinal pathologies.[1,5–7] In several animal models, the decreased inward K^+ conductance is accompanied by an alteration of the Müller cell response to osmotic stress. Müller cells from healthy retinae keep their volume constant in hypotonic solution for 15 minutes, whereas Müller cells from pathologically altered retinae of different species swell immediately.[8-11] However, until now responses of human Müller cells to osmotic stress have never been investigated. We previously described that gliotic alterations of electrophysiologic properties of Müller cells also occur in human retinae obtained from patients affected with various retinal diseases.[12,13] In the course of the present study, we had the opportunity to obtain human retina from eyes with uveal melanoma for physiologic experiments. Uveal melanomas arise from melanocytes in the choroid, ciliary body, and iris, and are the most common primary intraocular tumors in the adult population.[14] Although the origin of uveal melanoma is not in the retina itself, the retinal tissue is affected, for instance by induction of serous retinal detachment[15] and by secretion of cytokines from melanoma cells that activate retinal cells.[16] In Müller cells from eyes with melanoma, electrophysiologic alterations were demonstrable but less pronounced than those in cases of proliferative vitreoretinopathy or glaucoma.[5,12,17] Although Müller cells are assumed to be involved in the formation of retinal edema,[1,18] there are yet no data regarding the responses of Müller cells to osmotic stress in retinal slices from human eyes. Therefore, the aim of the present study was to investigate general responses of Müller cells from human eyes to osmotic stress and to prove whether Müller cells from human eyes affected with uveal melanoma show similar alterations of these responses as have been described before in several animal models. These experiments were accompanied by electrophysiologic recordings.

Materials and Methods

Human tissue was used in accordance with applicable laws and with the Declaration of Helsinki after approval by the ethics committee of the University of Leipzig, Faculty of Medicine. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. We used material derived from a total of 11 eyes that were enucleated from patients affected with uveal melanoma (termed "melanoma eyes"). After removal of the melanoma for histopathologic processing, retinal tissue from areas far away from

the melanoma was available within a few hours (in most cases <5) after enucleation. In the eyes of 8 patients the used retina appeared macroscopically normal. If any, retinal detachment from the retinal pigment epithelium could be observed only in restricted areas close to the melanoma. In 3 eyes containing extremely large uveal melanomas (at least 10-mm diameter) widespread retinal detachment was found. For control reasons, eyes from 9 postmortem organ donors without any reported eye disease were supplied after removal of the cornea within 5 to 24 hours after death. The retinal material derived from organ donors is referred to as controls. The age of the melanoma patients varied between 36 and 84 years, that of the controls between 23 and 71 years.

Immunohistochemistry

Isolated retinae were fixed in 4% paraformaldehyde (in PBS) for 2 hours. After several washing steps in PBS, the tissues were embedded in PBS containing 3% agarose (w/v), and 70-μm-thick slices were cut by using a vibratome. For double-labeling, the slices were incubated in PBS (containing 5% normal goat serum, 0.3% Triton X-100, 1% dimethylsulfoxide) for 2 hours at room temperature and, subsequently, in a mixture of primary antibodies overnight at 4°C. After washing in 1% bovine serum albumin, secondary antibodies were applied for 2 hours at room temperature. The following antibodies were used: mouse anti-GFAP (1:400; Sigma-Aldrich, Taufkirchen, Germany), rabbit anti-cellular retinaldehyde-binding protein (CRALBP; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-aquaporin-4 (1:200; Sigma-Aldrich), rabbit anti-Kir4.1 (1:200; Alomone Laboratories, Jerusalem, Israel), and secondary antibodies (goat anti-mouse Cy2-conjugated, 1:200; goat anti-rabbit Cy3-conjugated, 1:200; Jackson ImmunoResearch, Newmarket, UK). Cell nuclei were stained with a commercial dye (Hoechst 33258; Invitrogen, Karlsruhe, Germany). Control slices were incubated without primary antibodies; no unspecific labeling with secondary antibodies was observed (not shown). Images were taken with a laser scanning microscope (LSM 510 Meta; Zeiss, Oberkochen, Germany).

Electrophysiology

Procedures for cell isolation and electrophysiologic recordings were described elsewhere.[6] (All substances used were obtained from Sigma-Aldrich unless indicated otherwise.) Briefly, Müller cells were isolated by a combination of enzymatic treatment (0.2–0.4 mg/mL papain for 30 minutes at 37°C) and mechanical dissociation, isolated Müller cells were identified due to their morphology. Recordings were performed in the whole-cell configuration of the patch-clamp technique at room temperature. Isolated cells were suspended in extracellular solution (in mM): 135 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4, 10 N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES), 11 glucose, adjusted to pH 7.4 with tris(hydroxymethyl)aminomethane (Tris). Recording electrodes (borosilicate glass from Science Products, Hofheim, Germany) had resistances of 4 to 6 MΩ when filled with (in mM): 130 KCl, 10 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 ethylene glycol-bis(2-aminoethylether-N,N,N′,N′-tetraacetic acid, 10 HEPES-Tris, pH 7.1. For current and voltage recordings, a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA) and quality management software (ISO2; MFK, Niedernhausen, Germany) were used. Currents were low-pass filtered at 1 or 6 kHz and digitized at 5 or 30 kHz, respectively, using a 12-bit A/D converter. Current amplitudes were measured at the end of 250-ms voltage steps. The membrane potentials were recorded in the current-clamp mode. The membrane capacitance was measured by the integral of the uncompensated capacitive artifact evoked by a 10-mV step in the presence of 1 mM Ba^{2+}. Mean values with SDs are given; Student's *t*-test and Mann-Whitney *U* test were used for statistical analysis. Differences were considered significant at $P < 0.05$.

Müller Cell Swelling

To determine volume changes of Müller cells evoked by hypotonic stress, the cross-sectional areas of their somata were recorded in retinal slices. This method was described in detail elsewhere.[9] Briefly, 1-mm-thick retinal slices were placed in a recording chamber and loaded with a commercial vital dye (MitoTracker Orange, 1 μM; Invitrogen, Karlsruhe, Germany) to stain the Müller cells.[19] Afterward, their somata can be clearly identified in the inner nuclear layer (Fig. 1A). The extracellular solution contained (in mM): 136 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11 glucose, adjusted to pH 7.4 with Tris. The hypotonic solution (60% of control osmolarity) was made by adding distilled water. The chamber was continuously perfused with the respective solutions. The slices were examined with a laser scanning microscope (LSM 510) and a water-immersion objective (Achroplan 63x/0.9; Zeiss). The vital dye (MitoTracker Orange) was excited at 543 nm and emission was recorded with a 560-nm long-pass filter. During the experiment, the somata of 4 to 5 dye-stained Müller cells per slice were recorded at the plane of their largest extension by continuously adjusting the focal plane.

Results

Morphology of Human Müller Cells and Immunohistochemical Stainings

Müller cells take up fluorescent dyes more efficiently than other retinal cell types and can therefore easily be identified in freshly prepared slices (Fig. 1A).[19] In addition, we used fixed retinal tissue for immunohistochemistry. As shown in Figures

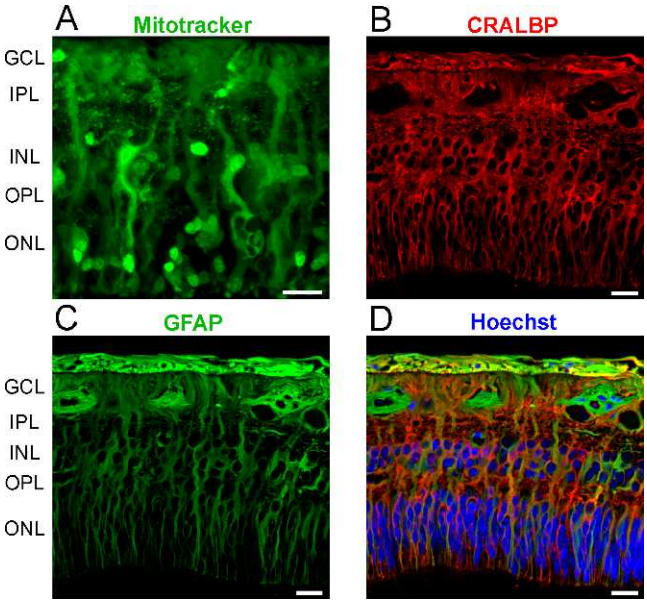

Figure 1. Morphologic characterization of Müller cells in human retinal slices. (**A**) Incubation of a freshly prepared slice with fluorescent dye (Mitotracker Orange) resulted in a preferential staining of Müller cells. The recordings of Müller cell somata in the inner nuclear layer (INL) in swelling experiments were performed in such preparations. (**B–D**) Immunohistochemical staining of a retinal slice from a melanoma eye without widespread detachment. Müller cells were positive for cellular retinaldehyde-binding protein (CRALBP, **B**). Immunoreactivity for GFAP was found in Müller cells throughout the retina (**C**). (**D**) Merged picture of CRALBP (*red*) and GFAP (*green*) staining. Double-labeled structures appear in *yellow*. Cell nuclei were counterstained with commercial dye (Hoechst 33258). GCL, ganglion cell layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Scale bars, 20 μm.

1B–D, structures positive for the Müller cell marker CRALBP were found to express GFAP (yellow in Fig. 1D). Because expression of GFAP is known to be upregulated in gliotic Müller cells,[3,4] this observation suggests that reactive Müller cell gliosis is induced in the eyes of patients affected with uveal melanoma.

In animal models of retinopathies, downregulation of functional Kir4.1 channels was implicated in the induction of osmotic Müller cell swelling.[1,2,8] We determined the retinal distribution of Kir4.1 and the glial water channel, aquaporin-4, by immunohistochemical labeling of retinal slices from a control and a melanoma eye (complicated by widespread retinal detachment). As shown in Figure 2, Kir4.1 labeling in the control was preferentially localized to the perivascular space and to the microvilli of Müller cells extending into the subretinal space. In the slice from the melanoma eye, the perivascular Kir4.1 labeling was absent (Fig. 2). Aquaporin-4 was predominantly localized to the innermost retinal layers and in perivascular glial membrane sheets (Fig. 2). There was no difference in the distribution of aquaporin-4 between tissues from the control and melanoma eyes (Fig. 2). Although the results must be confirmed in future experiments, the data suggest that uveal melanoma accompanied by retinal detachment may be associated with downregulation of Kir4.1 in perivascular Müller cell membranes. A similar downregulation of perivascular Kir4.1 and lack of alteration of aquaporin-4 distribution was previously described in a rat model of retinal ischemia-reperfusion.[8]

Electrophysiologic Characterization of Müller Cells

Typical current patterns evoked by depolarizing and hyperpolarizing voltage steps are shown in Figure 3A. The inward currents were mainly mediated by Kir channels because they could be largely blocked by addition of Ba^{2+} (0.3 or 1 mM; data not shown). Mean values for inward current amplitudes, membrane potentials, and membrane capacitances are given in detail in Table 1. Inward current amplitudes were decreased in both groups of patients with melanoma, with the effect being stronger in the group with widespread detachment. However, the fact that cells from one of the less severely affected melanoma eyes displayed inward currents of only 154 ± 162 pA demonstrates that widespread retinal detachment is not the sole cause of strong inward current decrease. Because functional Kir4.1 channels are important for the maintenance of the membrane potential,[20] the decrease of the inward currents was accompanied by a decrease of the membrane potential, particularly in cells from eyes with widespread detachment (Fig. 3B). Müller cells depolarize when their inward current amplitude is decreased below approximately 300 pA.

Osmotic Müller Cell Swelling

Earlier we have shown that normal Müller cells from rodents keep the volume of their somata constant under hypotonic conditions, whereas Müller cell swelling was induced in pathologically altered retinae.[21] Here, we investigated for the first time the response of human Müller cells to osmotic stress. Retinal slices were used from 4 control eyes and 9 melanoma eyes, including the 3 melanoma eyes with widespread retinal detachment. The absolute cross-sectional areas of Müller cell somata recorded in isotonic solution do not differ significantly between control cells (64.5 ± 11.3 μm^2, $n = 32$) and cells from melanoma eyes with (64.2 ± 16.7 μm^2, $n = 20$) and without widespread detachment (69.6 ± 32.2 μm^2, $n = 49$). In the following, the values recorded under hypotonic conditions are given as a percentage of the soma area in isotonic solution. The data are summarized in Figure 4 and Table 2. Superfusion with hypotonic solution for 4 minutes did not cause a significant increase in the size of Müller cell somata in slices from control eyes (101.7 ± 1.2%, $n = 32$ cells) when compared with the soma size measured in isotonic solution (i.e., 100%). By contrast, Müller cells in retinal slices from melanoma eyes increased their soma size to 109.5 ± 3.1% ($n = 61$ cells from 9 eyes, $P < 0.001$ compared with the value from controls). Moreover, the 20 cells that were recorded from eyes with very large tumors and widespread retinal detachment displayed an even stronger swelling to 117.0 ± 5.1%. This is significantly

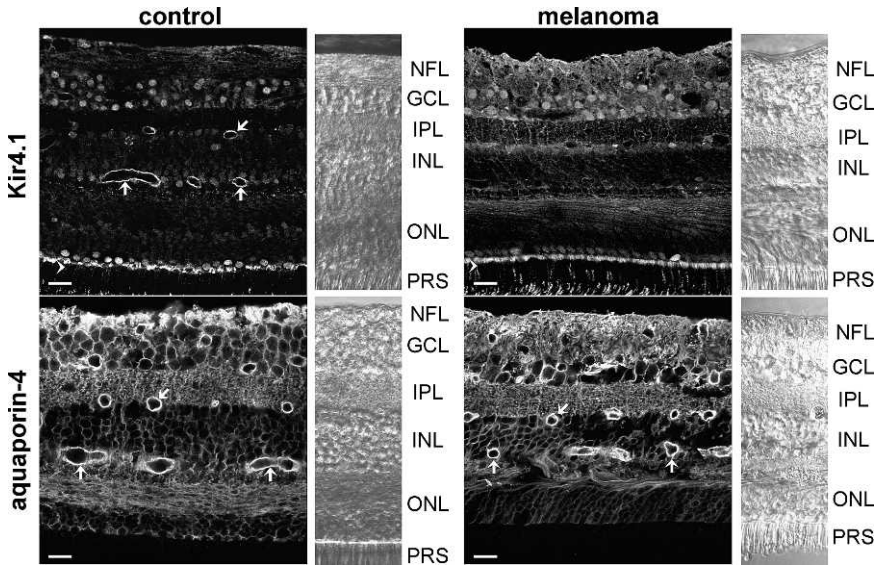

Figure 2. Immunolabeling of human retinal slices against Kir4.1 (*above*) and aquaporin-4 (*below*). The slices were derived from a control eye (*left*) and a melanoma eye (with widespread retinal detachment; *right*). *Arrows*, perivascular staining; *arrowheads*, staining of Müller cell's microvilli. Note the lack of perivascular Kir4.1 labeling in the tissue from the melanoma eye. Differential interference contrast images show the general retinal structure. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NFL, nerve fiber layer; ONL, outer nuclear layer; PRS, photoreceptor segments. Bars, 20 μm.

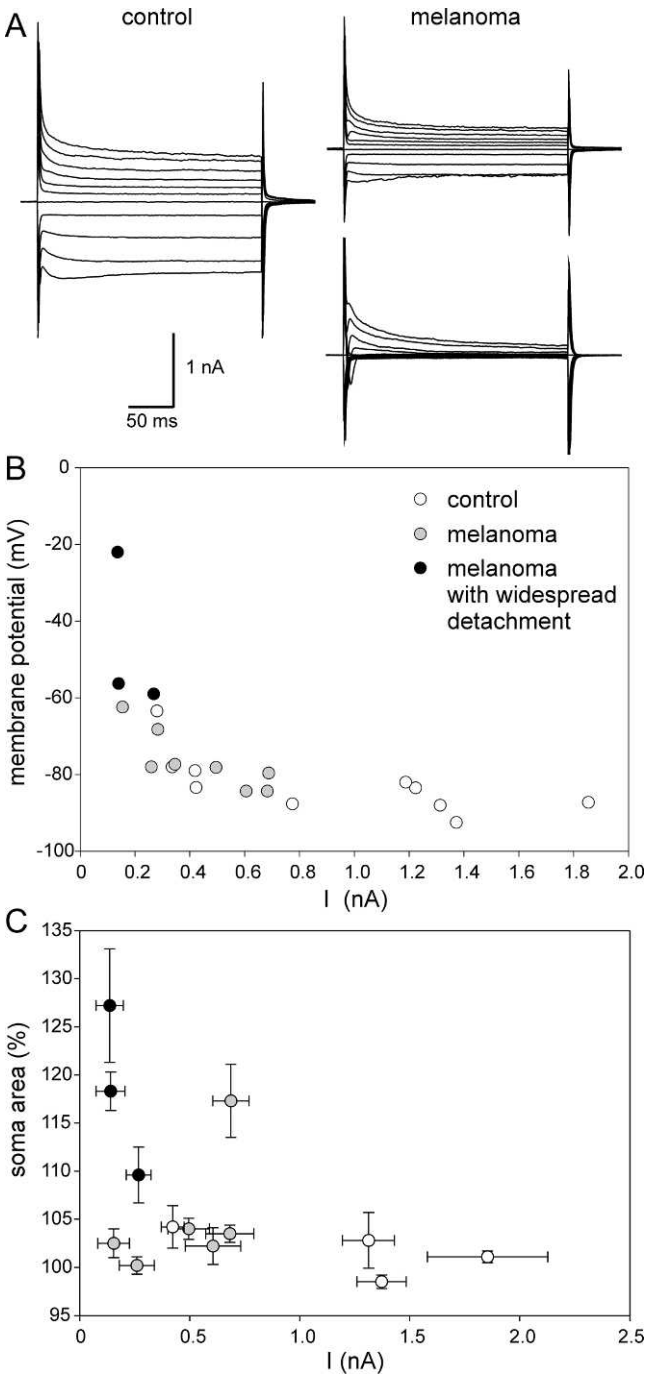

Figure 3. Electrophysiologic properties of human Müller cells. (**A**) Representative current traces of a cell from a healthy control and of cells from two patients with uveal melanoma. The *bottom traces* display currents of a cell from a melanoma eye with widespread retinal detachment. Voltage steps were applied from a holding potential of −80 mV to potentials between −160 and +40 mV (20-mV increment). The dominant inward currents elicited in the control cell are clearly reduced or even lacking in the cells from melanoma eyes. The transient inward currents are supposed to be mediated by voltage-dependent Na^+ channels. (**B**) Relationship between inward K^+ current amplitudes and membrane potentials in human Müller cells. The inward current amplitudes were recorded at the 60-mV hyperpolarizing step (i.e., at a potential of −140 mV). Each symbol represents the mean value of the recorded cells from one melanoma patient (*black* and *gray symbols*) or one control eye (*white symbols*). The current amplitudes display a large variability with all values from melanoma eyes being <800 pA. Very small current amplitudes (<300 pA) were associated with decreased membrane potentials. (**C**) Relationship between inward current amplitudes and soma areas recorded in swelling experiments. In some cases small inward current amplitudes were associated with osmotically induced swelling. Mean values with SEs for individual melanoma patients (*black* and *gray*) or controls (*white*) are shown.

different from the 41 cells from retinal tissues of the remaining 6 melanoma eyes (105.8 ± 2.5%, $P < 0.01$). However, the mean soma area of the cells from eyes without widespread detachment is still significantly larger than the control value, indicating that the uveal melanoma caused an alteration of Müller cell swelling (columns 5 and 1 in Fig. 4).

As demonstrated previously, Müller cell swelling can also be induced by hypotonic solution in the presence of the Kir channel blocker Ba^{2+}.[8] We applied Ba^{2+} (1 mM) onto retinal slices from control eyes ($n = 29$ cells) and from melanoma eyes ($n = 50$ cells from 8 eyes) and observed significant Müller cell swelling to 117.5 ± 2.8% and 119.4 ± 1.7%, respectively ($P < 0.001$ compared with the values without Ba^{2+}; Fig. 4).

It was shown in a number of pharmacologic experiments that Müller cell swelling can be induced by oxidative stress and by inflammatory lipid mediators such as arachidonic acid and prostaglandins, and can be decreased by substances with antiinflammatory effects.[22] Here, some of these substances were applied to retinal tissue from human eyes. We used material from 2 melanoma eyes with widespread retinal detachment. Müller cells in the respective slices swelled in hypotonic solution. To investigate swelling-reducing pharmacologic effects, swelling of Müller cells in retinal slices from 2 other patients with melanoma and from a control eye was induced by additional application of Ba^{2+} or arachidonic acid. The results are given in detail in Table 2. In summary, it is shown that antiinflammatory substances and a reducing agent diminish the swelling induced by hypotonic solution, hypotonic solution with Ba^{2+}, or hypotonic solution with arachidonic acid.

Cells from all eyes used in swelling experiments were also used for patch-clamp recordings. This allowed correlation of the inward K^+ current amplitudes with osmotically induced swelling (Fig. 3C). Although we failed to find a significant linear correlation between both data sets, the data show that significant swelling could be induced only in cells displaying inward current amplitudes of less than 700 pA. However, there were also some cells with very small currents that did not swell significantly (Fig. 3C). It can be concluded that the reduction of inward K^+ currents is associated with an increased vulnerability to osmotic stress in Müller cells from melanoma eyes with widespread retinal detachment.

Discussion

Uveal melanomas are relatively rare events, but may be life-threatening because of the formation of metastases outside the eye. Standard treatments include the removal of the tumor by enucleation in cases of larger tumors, whereas smaller ones might be treated by photocoagulation, thermotherapy, radiotherapy, and other approaches.[23] In cases of enucleation, human retina could be obtained shortly after the operation and, thus, could be used in physiologic experiments. GFAP expression and decreased inward currents have been described as hallmarks of Müller cell gliosis.[1,4] Both alterations were observed in cells from melanoma eyes. Therefore, the retinae from melanoma-affected eyes gave the opportunity to study responses of gliotic human Müller cells to osmotic stress. The data may help to understand similar alterations of reactive glial cells in general.

Table 1. Electrophysiologic Properties of Human Müller Cells

	Control	Melanoma without Widespread Detachment	Melanoma with Widespread Detachment
n	54 cells from 9 donors	53 cells from 8 patients	18 cells from 3 patients
I	978 ± 651 pA	479 ± 315 pA***	191 ± 151 pA***•••
E	−82 ± 11 mV	−78 ± 14 mV	−48 ± 22 mV***•••
C	80 ± 30 pF	82 ± 22 pF	95 ± 22 pF

Mean values (with SDs) are given. I, inward current amplitude recorded at a hyperpolarizing step from −80 to −140 mV; E, membrane potential recorded in the current-clamp mode; C, membrane capacitance recorded in the presence of 1 mM Ba^{2+}.

Significant difference compared with the control value: ***$P < 0.001$. Significant difference compared with the value from melanoma eyes without widespread detachment: •••$P < 0.001$.

We present here, for the first time, data regarding the volume regulation of Müller cells in retinal slices from human eyes under osmotic stress conditions. Müller cells are supposed to prevent detrimental shrinkage of the extracellular space during neuronal activity, by inhibiting cellular swelling even in hypotonic extracellular solution.[21,24] This has been observed in retinal slice preparations of rats,[8] mice,[25] and pigs.[9] These studies also demonstrated that Müller cell swelling under hypotonic conditions correlated with a reduced inward K^+ conductance that occurs in a variety of animal models for retinal pathologies (and can be induced by application of Ba^{2+}). Using human retinal tissue, we confirmed the data from animal models; control Müller cells did not show significant swelling in hypotonic solution, whereas Müller cell swelling was observed in retinal slices from some melanoma eyes. The swelling was particularly strong when the uveal melanoma had caused a widespread retinal detachment. A similar observation was made in regard to the inward current amplitudes. Müller cells from melanoma eyes with widespread retinal detachment all displayed small inward current amplitudes (<300 pA). We had recorded inward currents and/or membrane resistances of Müller cells from melanoma eyes in earlier studies, and found a considerable variability of these values.[12,17] The electrophysiologic data are in agreement with the immunohistochemical data, which demonstrated reduced Kir4.1 immunoreactivity in the retina of a melanoma eye.

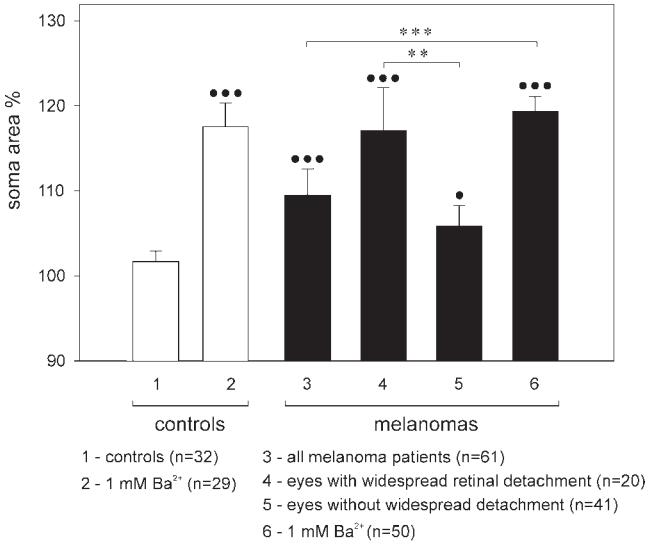

Figure 4. Osmotic swelling characteristics of human Müller cells. The soma area of Müller cells in retinal slices was recorded 4 minutes after application of a hypotonic solution. Mean values with SEs are shown. Numbers of recorded cells are given in parentheses. See text for additional details. •$P < 0.05$, •••$P < 0.001$, compared with the mean value of healthy controls (i.e., column 1). **$P < 0.01$, ***$P < 0.001$.

Generally, our data confirm that pathologic alterations caused by uveal melanoma and/or retinal detachment may alter certain physiologic properties of retinal Müller cells, such as the amplitude of inward currents and the volume responses to osmotic stress. At present, it is difficult to discriminate between primary effects of the uveal melanoma itself and secondary effects of the melanoma-induced detachment. The occurrence of stronger pathologic alterations in cases with a melanoma-associated widespread retinal detachment might be caused by processes not directly dependent on the tumor. Retinal detachment impairs the contact to the choroidea, causing ischemia-like conditions at least for the outer retina.[26] The prolonged retinal circulation times observed after retinal detachment may result in hypoxia also in the inner retinal tissue.[27] The exact cause–effect relation remains to be determined in further studies.

There are a few studies dealing with Müller cell reactions in melanoma eyes. For example, Ghazi-Nouri et al.[28] immunostained human retinal slices for GFAP. Although in the control retina from an organ donor GFAP was found only in the

Table 2. Pharmacologic Characterization of Müller Cell Responses to Osmotic Stress

Patient	Condition	Soma Area (%)	*n* (cells)	*P*
1. Melanoma	Hypotonic	127.2 ± 5.9	4	••
	Hypotonic + bromo	99.9 ± 1.3	5	*
	Hypotonic + DTT	103.1 ± 2.0	6	**
2. Melanoma	Hypotonic	118.3 ± 2.0	9	•
	Hypotonic + triam	103.1 ± 1.5	10	**
3. Melanoma	Hypotonic + Ba^{2+}	113.8 ± 1.2	5	••
	Hypotonic + Ba^{2+} + bromo	102.0 ± 1.2	6	**
	Hypotonic + Ba^{2+} + DTT	102.9 ± 1.2	6	**
	Hypotonic + Ba^{2+} + indo	99.1 ± 0.8	5	**
4. Melanoma	Hypotonic	104.3 ± 1.1	7	
	Hypotonic + AA	116.9 ± 2.6	7	•
	Hypotonic + AA + triam	104.4 ± 1.7	8	*
	Hypotonic + Ba^{2+}	124.7 ± 1.4	5	••
	Hypotonic + Ba^{2+} + triam	108.2 ± 3.3	3	*
5. Control	Hypotonic	102.8 ± 2.9	7	
	Hypotonic + AA	116.9 ± 2.8	6	•

Mean values (with SEs) are given. A widespread retinal detachment was observed in melanoma patients 1 and 2. Müller cells from these patients swelled in hypotonic solution. AA, arachidonic acid (10 μM); bromo, 4-bromophenacyl bromide (300 μM), phospholipase A_2 inhibitor; DTT, dithiothreitol (3 mM), reducing agent; indo, indomethacin (10 μM), cyclooxygenase inhibitor; triam, triamcinolone (100 μM), antiinflammatory glucocorticoid.

Significant swelling of Müller cell somata induced by hypotonic solution, by hypotonic solution with Ba^{2+}, or by hypotonic solution with arachidonic acid, respectively (as compared with isotonic solution): •$P < 0.05$, ••$P < 0.01$. Significant decrease of cellular swelling: *$P < 0.05$, **$P < 0.01$.

ganglion cell layer (and, thus, in astrocytes and possibly in Müller cell endfeet), Müller cells were immunopositive for GFAP throughout all layers of the retinae from melanoma eyes. We could confirm GFAP expression in Müller cells in the retina of a melanoma eye. Similar alterations were observed by Enzmann et al.[16] in retinal explant cultures: Soluble factors from melanoma cells caused a disorganization of the retinal structure and an increase in GFAP immunoreactivity in Müller cells. Moreover, the formation of cystoid macular edema in the retina of eyes enucleated from patients with uveal melanoma has been described.[29,30] Electron microscopical investigation displayed widespread swelling and necrosis of Müller cells. The authors suggested that intracellular water accumulation in Müller cells, resulting in cytotoxic edema, was the main pathogenic factor contributing to the development of macular edema. Green and Robertson[31] also observed Müller cell swelling, although the investigated melanoma eye had an additional photic retinopathy. These studies are in agreement with the present data, which demonstrate that Müller cells from melanoma eyes with retinal detachment may lose the ability to keep their volume constant under hypotonic conditions. Interestingly, it has been observed that retinal expression of vascular endothelial growth factor (VEGF) is increased in melanoma eyes, and also in Müller cells that were not directly in contact with tumor cells.[32,33] As we have demonstrated earlier, one of the effects of VEGF is the inhibition of osmotically induced Müller cell swelling by activation of an intrinsic signaling cascade.[34] It might be speculated that a swelling-inhibitory effect of VEGF may represent a positive side effect of increased expression of this factor in melanoma eyes.

In summary, our data on human cells convincingly confirmed the knowledge obtained from animal models of reactive gliosis. Moreover, they confirm earlier data from animal models demonstrating that Müller cell swelling is induced by inflammatory lipid mediators, and is suppressed by antiinflammatory substances. Increases of lipid peroxides and of inflammatory parameters in the retina of melanoma eyes have been demonstrated.[35] Our study encourages further experimental effort to understand the pathophysiology of Müller cells in more detail, hopefully leading to novel therapeutic approaches that specifically target Müller cells in future attempts to treat retinal diseases associated with edema formation.

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