Induction of vitronectin and integrin αv in the retina after optic nerve injury

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Purpose: Vitronectin is a secreted glycoprotein present in blood plasma and is present in the extracellular matrix of many tissues. It was found in the retinal cDNA library that contains genes whose expression is upregulated after optic nerve injury in a previous study. The purpose of this study was to assess the temporal and spatial changes in expression of vitronectin and integrin αv in the retina following optic nerve injury.

Methods: Adult Balb/c mice underwent crush of the optic nerve in one eye only. RT-PCR was used to determine the temporal expression of vitronectin mRNA in the retina after injury. In addition, expression at the protein level in the retina and the optic nerve of vitronectin and its major receptor subunit, integrin αv, was analyzed by immunohistochemistry.

Results: Upregulation of vitronectin mRNA in the retina was detected at one day after injury, peaking at three days, and maintained up to one week. An elevated expression of vitronectin protein was also observed in the inner retina, optic nerve head, and the optic nerve after nerve crush. In the inner retina, the increased expression of vitronectin was found in retinal ganglion cells (RGCs) and its surrounding extracellular matrix. Expression of integrin αv was also increased in the RGC layer and in the glial cells of the nerve head and the crush site.

Conclusions: As vitronectin is an extracellular protein that can support cell attachment and promote neurite extension, elevated expression of vitronectin and its receptor may facilitate axonal regeneration following injury. We propose that treatment sustaining secretion of endogenous vitronectin or direct application of exogenous vitronectin may be a method to augment regeneration of the severed optic axons.

Vitronectin is a secreted glycoprotein present in blood plasma and is present in the extracellular matrix of many tissues [1]. It is a multiadhesion molecule and interacts with extracellular glycosaminoglycans, collagen, and other extracellular proteins [2]. Moreover, through the RGD sequence, vitronectin can bind integrins on the cell surface, thus connecting cells to matrix proteins [3]. Circulating vitronectin is synthesized in the liver, and its concentration in the blood plasma is upregulated during systemic inflammation [4]. However, significant amounts of vitronectin mRNA have also been detected in many other tissues, such as brain and heart, indicating that vitronectin can be produced locally [4,5].

In addition to being a pivotal regulator in the complement, fibrinolytic, and coagulation systems, vitronectin plays a number of roles in the retina. Vitronectin mRNA is detected in the neural retina, including photoreceptor cells and retinal ganglion cells (RGCs) [6], and in the retinal pigment epithelium (RPE) [7]. The integrin αvβ5 vitronectin receptor is also expressed on the apical membrane of human RPE. This vitronectin receptor participates in the binding of photoreceptor rod outer segment during phagocytosis by cultured human RPE [8]. Moreover, in age-related macular degeneration, vitronectin is a major component of the distinct extracellular deposits that accumulate below the RPE on Bruch’s membrane and is suspected as a possible causative factor that results in the compromised central vision [9]. Several lines of evidence also suggest that vitronectin influences neurite outgrowth of developing retinal cells. Vitronectin is expressed in the chick eye from embryonic day 5 (E5) [10]. Expression of vitronectin receptors, αvβ3 and αvβ5, is also detected in developing chick retina [11]. Cultured embryonic chick retinas attach and extend neurites on vitronectin-coated dishes [12]. Furthermore, purified chick RGCs from E10 chick embryo grow better on vitronectin than on laminin [13]. These results show that vitronectin can support survival and neurite outgrowth of differentiating retinal neurons [10]. However, the effects of vitronectin on adult neural retina are still not clear.

In adult mammals, severed axons within the optic nerve may show a transient local sprouting but do not regrow over long distances [14]. This failure of regeneration is usually attributed to the formation of a gliotic scar [15], the presence of extracellular inhibitors [16-20], intrinsic changes in the gene expression of mature neurons [21,22], and massive apoptosis of damaged neurons [23-25]. By neutralizing hostile factors, potentiating neuronal outgrowth ability, and maintaining cell survival, one may be able to facilitate functional recovery of visual pathways [26]. Although many studies have demonstrated how to counteract inhibitory molecules and support neuronal survival [27-29], few investigations have focused on intrinsic gene expression necessary for neuronal regeneration [30,31].
Previously, we looked for genes that may behave in a similar manner as GAP43, which is known to augment neuronal regeneration after injury [32,33], using a subtractive hybridization approach [34]. Several genes that are upregulated in the retina following optic nerve injury at different time points were identified [34]. Among these genes, vitronectin was detected with a high frequency in the subtractive libraries. Here, we used reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry to further explore the expression pattern of vitronectin throughout the injured retina. The expression of one of its receptor subunits, integrin αv [35-38], was also analyzed. We find that after optic nerve crush both proteins are induced in RGCs and in glial cells that are present in the nerve head and at the injured site.

METHODS

Animals and surgery: Adult Balb/c mice that were at least two months old and weighing over 25 g were obtained from the animal facility of National Yang-Ming University. The experimental protocol was approved by the National Yang-Ming University Animal Care and Use Committee. Institute guidelines were followed on handling of animals. Mice were given food and water ad libitum. For the optic nerve crush, mice were anesthetized by injecting a combination of ketamine (0.1 mg/g body weight) and xylacaine (0.03 mg/g body weight) intraperitoneally. Surgery for all mice was conducted by the same surgeon under sterile conditions using a stereomicroscope. A conjunctival incision was made over the dorsal aspect of one eye, which was then gently rotated downward in the orbit. The orbital muscles were teased and deflected aside to expose the optic nerve at its exit from the globe. A jewelers forceps was used to crush the optic nerve twice across the entire width near the back of the eye (within 0.5 mm). Care was taken not to damage the ophthalmic artery and retrolubar sinus. The eye was then rotated back into position and rinsed with sterile saline. Mice were allowed to recover. The contralateral eye without surgery was used as control. At different time points (one day, three days, and seven days) after surgery, the retina and the attached nerve were dissected out for analysis.

Reagents: Superscript reverse transcriptase II (RTase) was purchased from Invitrogen (Carlsbad, CA). dATP, dCTP, dGTP, and dTTP were obtained from Roche (Mannheim, Germany). RNase inhibitor (RNasin) and Taq DNA polymerase were purchased from Promega (Madison, WI). Primers were synthesized by MDBiol (Taipei, Taiwan). All other chemicals, unless specified, were purchased from Merck (Darmstadt, Germany).

RT-PCR and quantitative image analysis: Total RNA of five retinas from each group at each time point was prepared using RNeasy Mini kit according to the user manual (Qiagen, Valencia, CA). Total RNA was denatured at 75 °C for 5 min. Reverse transcription was performed in a total volume of 20 µl containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM dNTP, 0.5 µg of oligo dT, 20 U of RNasin, and 100 U of RTase for 1.5 h at 50 °C. Samples were stored at -20 °C until use. Primers used for PCR amplification were as follows: for GAPDH (NM_008084, from nucleotide position 565 to 1006), ACC ACA GTC CAT GCC ATC AC and TCC ACC ACC CTG TTG CTG TA; for vitronectin (NM_011707, from nucleotide position 114 to 498), TCC GTT GAT CAG TGG TGT CGG and CTG GCT GAC CAA GAG TCA TGC. The expected size of PCR fragment for GAPDH is 452 bp and for vitronectin is 385 bp. Amplification of cDNA was performed in a GeneAmp 9700 (Applied Biosystems, Foster City, CA) with each tube containing a final volume of 50 µl, consisting of 1 µl of cDNA, 1X PCR buffer, 1.25 U of Taq DNA polymerase, 0.25 mM dNTP, and one set of primers (200 nM). Samples were covered with 15 µl of mineral oil and were first denatured at 94 °C for 30 s, then cycles of 94 °C for 5 s, 56 °C for 15 s, 72 °C for 30 s were performed. Reaction was paused at each cycle after cycle numbers 23 to 27, and 8 µl of PCR products were retrieved before resumption of the PCR. Samples were analyzed on a 2% agarose gel, and DNA was visualized with ethidium bromide staining and UV illumination. Gel intensity after cycle 25 was analyzed using the ImageQuant software (version 1.1, Molecular Dynamics, Sunnyvale, CA). A scanning area was chosen around the target band on the gel, and an area of the same size was used for each sample analyzed at the various time points. The local median was selected as the method for background correction. The volume for each target band was measured and expressed as the ratio relative to GAPDH. Statistical analysis was performed with the paired t-test.

Immunohistochemistry: The injured eye and control eye were enucleated at one day, three days, and one week postsurgery (four mice for each time points), and maintained in Hank’s solution. After the anterior segments were removed, posterior eyecups were fixed in 4% paraformaldehyde solution for 1 h at room temperature. The eyeballs were transferred to 30% sucrose to sit overnight, were then submerged in Tissue-Tek OCT media (Sakura Finetechical Co., Tokyo, Japan) before being frozen on dry ice. Sections were cut at 12 µm thick. Crushed and control retina sections were collected on the same slides (SuperFrost, Fisher Scientific, Pittsburgh, PA).

For staining, sections were fixed with 4% paraformaldehyde solution for one h at room temperature, washed with phosphate buffer solution (PBS)/0.1% Tween for 15 min at room temperature, followed by PBS for 15 min twice. Blocking was done with 20% normal goat serum or 5% bovine serum albumin (BSA) for 1 h at room temperature, and then the sections were stained with rabbit antivitronectin antiserum (Biotrend, Cologne, Germany) or anti-integrin αv antiserum (Chemicon, Temecula, CA), and antineurofilament monoclonal antibody (heavy, 200 kDa, clone RT 97, Roche) diluted in 3% normal goat serum or 5% BSA at 4 °C overnight. Sections blocking with 20% normal goat serum or 5% BSA gave similar results. The rabbit antivitronectin antiserum recognizes mouse vitronectin but does not recognize goat vitronectin. One set of slides without primary antibody was used as control in each experiment. Sections were then washed with PBS three times and incubated with fluorescein-conjugated goat antirabbit antibody and rhodamine-conjugated goat antimouse antibody.
(Cappel, Aurora, OH) at room temperature for 2 h. For nuclear staining, 4',6-diamidino-2-phenyindole (DAPI, 0.16 µg/ml) was added for 15 min at room temperature, and washed with PBS three times. Sections were analyzed with a Nikon Diaphot inverted microscope (E300, Nikon, Tokyo, Japan) equipped with a MicroMax cool CCD (Princeton Instrument, Trenton, NJ). Image acquisition was performed using MetaMorph software (version 4.5r6, Universal Image Corporation, Downingtown, PA) with individual filter sets for each channel and were assembled with Adobe PhotoShop (version 7.0, Adobe Systems, San Jose, CA).

RESULTS

Induction of vitronectin mRNA after optic nerve injury: To pursue whether vitronectin mRNA expression in the retina is elevated after damage in the optic nerve, a semiquantitative RT-PCR assay was employed to measure amount of vitronectin mRNA in the retina. A representative gel of this analysis is shown in Figure 1A. The expression levels of vitronectin relative to those of GAPDH were then calculated using the ImageQuant software. The statistical analysis of three independently prepared RNA sets was performed and is shown in Figure 1B. The results reveal that expression of vitronectin had increased by two fold on day one and day three after optic nerve crush in comparison with the control samples. Although the induction of vitronectin mRNA was still visible at seven days after injury, this increase was not statistically significant.

Vitronectin and integrin αv expression in normal eye: To characterize whether the increase in vitronectin mRNA expression is accompanied with synthesis of vitronectin protein and to clarify whether the effect is in the RGCs, we first examined the expression pattern of vitronectin in the normal eye using antivitronectin antiserum. The expression pattern of the vitronectin receptor was also investigated using anti-integrin αv antiserum, since integrin αv is the major α subunit of vitronectin receptors [35-38]. In the normal eye, vitronectin was found in RPE, inner segments of photoreceptors, the outer plexiform layer, and the inner retina (Figure 2D). A diffuse expression pattern of vitronectin was observed, which may indicate the extracellular distribution of secreted vitronectin.

In the inner retina, the staining for vitronectin was present in RGCs and in the extracellular matrix that surrounds the RGCs (Figure 2F,G). The retinal ganglion layer was identified by the DAPI nuclear staining (Figure 2G). To outline the location of nerve fiber layer in the retina, we labeled the sections with an antineurofilament monoclonal antibody. Neurofilament staining revealed the axonal fibers in the nerve fiber layer, which represent the projection of the RGCs (Figure 2C, J). The merged picture (Figure 2E) showed the vitronectin expresses over the nerve fiber layer in addition to the RGC layer.

Figure 1. Increase of vitronectin mRNA expression in the retina after optic nerve crush. A: A semi-quantitative RT-PCR was adopted to examine the expression of vitronectin mRNA in the retina. RNA of both crushed and control retina at one day, three days, and seven days after crush was reverse-transcribed to cDNA and used as the template for PCR. Expression of GAPDH was used as an internal control. PCR products were removed after each cycle from cycles 23 to 27. B: Gel intensity (A) was analyzed using the ImageQuant software. The relative amount of vitronectin mRNA was expressed as ratios to that of GAPDH. Closed columns represent gel intensities of crushed groups and open columns represent those of control groups. Statistical analysis was performed with a paired t-test. Bars in graph represent means; the error bars represent the standard deviation. Both one day and three day groups revealed a statistically significant difference between the crushed and control retina (asterisk; p<0.05). Although the seven-day group also showed a similar trend, it was not statistically significant.
Figure 2. Expression of vitronectin and integrin αv in the retina and the optic nerve head. Sections of normal retina (A-N) and optic nerve head (O-X) were reacted with anti-vitronectin antiserum (VTN; D,F,R, green), anti-integrin αv antiserum (Intαv; K,M,W, green), anti-neurofilament antibody (NF; C,J,Q,V, red) and DAPI (B,I,P, blue); merged photomicrographs are also shown (E,G,L,N,S,X). Differential interference contrast light microscopic views are shown in A,H,O,T. Vitronectin is expressed in the nerve fiber layer and retinal ganglion cell (RGC) layer, inner and outer plexiform layer, inner segment of photoreceptor (PC), and retinal pigment epithelium (RPE). The nerve fiber layer is revealed by neurofilament staining. Immunostaining of integrin αv is present in inner retina, inner and outer nuclear layers, outer plexiform layer, inner segment of photoreceptors, and retinal pigment epithelium. The staining pattern of integrin αv in the RGC layer is seen in high magnification images (F,G and M,N from D and K, respectively). The inner nuclear (IN) layer is also identified. In the normal optic nerve head, vitronectin and integrin αv are weakly expressed. Rows of glial cells, indicated by DAPI staining, are found in the septum (between the nerve bundles) of the optic nerve head. The scale bars represent 50 µm in A,E,H,L; 25 µm in F,G,M,N; and 100 µm in O-X.
We also examined the expression of integrin αv in retina. It was observed in the RGC layer, inner and outer nuclear layers, the outer plexiform layer, inner segments of photoreceptors, and RPE (Figure 2K). Staining of integrin αv was detected on each cell in the RGC layer with a membrane-staining pattern (Figure 2M, arrows). In control, experiments which were performed without adding primary antibodies, no staining was observed in the inner retina (data not shown). As displaced amacrine cells are present in the RGC layer, we can not exclude the possibility that some immunopositive cells in the RGC layer were actually amacrine cells.

The expression patterns of vitronectin and integrin αv in the optic nerve head were investigated. Optic axon fibers originating from the nerve fiber layer converge on the optic nerve head and exit the eyeball through the scleral canal. Within the optic nerve head, the nerve bundles proceed in parallel and were stained with the antineurofilament antibody (Figure 2Q,V). In between the nerve bundles, columns of glial cells could be identified with DAPI staining (Figure 2P,U). The vitronectin expression in exiting axon fibers was much weaker than that of the inner retina layer (Figure 2R), which could be due to less accumulation of vitronectin in the exiting axons. A similar pattern of integrin αv expression was also present in the optic nerve head (Figure 2W).

**Increase of vitronectin and integrin αv expression in the retina after nerve crush:** When the optic nerve was subjected to crush injury, the vitronectin expression became elevated at the inner retina (Figure 3). The RGC layer and nerve fiber layer can be clearly recognized with antivitronectin antibodies after optic nerve crush. The induction of vitronectin was present on day one after crush, culminated at day three, and was maintained at day seven. Surprisingly, induction of integrin αv was also observed in the inner retina using antibodies against integrin αv (Figure 3). Expression of integrin αv was elevated in RGCs from one day to one week after crush. This increase of expression was unique for integrin αv, as no upregulation of α5 which binds fibronectin but not vitronectin was detected (data not shown). Among three integrin β subunits that bind vitronectin, β5 is upregulated in the RGC layer at day three after optic nerve injury (data not shown), suggesting the possibility that vitronectin can have effects on RGC by interacting with αvβ5.

**Expression of vitronectin and integrin αv in the optic nerve:** The differences of vitronectin and integrin αv expression between the crushed and control optic nerves were also examined. After optic nerve injury, the expression of vitronectin had increased at the optic nerve head at day one (data not shown) and on day three post-crush (Figure 4F). Simi-

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Figure 3. Increased expression of vitronectin and integrin αv in the retina after optic nerve crush. Section of crushed (B,D,F,H,J,L,N,P,R,T,V,X) and contralateral control (A,C,E,G,I,K,M,O,Q,S,U,W) retinas were stained with antivitronectin antiserum (A-L), anti-integrin αv antiserum (M-X), and DAPI (G-L,S-X) at one day, three days, and seven days after optic nerve crush. Induction of vitronectin and integrin αv was found within the retinal ganglion cell (RGC) layer in the crushed group at 1 day to 1 week postcrush (B,D,F,N,P,R, arrows), compared with the control retina. The inner plexiform layer (IPL) and inner nuclear layer (INL) are identified. The scale bar represents 25 μm.
Figure 4. Increased expression of vitronectin and integrin αv over optic nerve head and optic nerve.

A-P: Sections of crushed (B.D.F.H.J.L.N.P) and contralateral control (A.C.E.G.I.K.M.O) optic nerve heads were stained with anti-vitronectin antiserum (VTN; A.B.E.F), anti-integrin αv antiserum (Intαv; I.J.M.N), and DAPI (C.D.K.L) at three days after optic nerve crush. High power fields (C-H.K-P) of the optic nerve head region (from the rectangles in A.B.I.J) are presented in each column with merged micrographs (G.H.O.P) below. Induction of vitronectin was found in the optic nerve head (F.H, arrows) and increased expression of integrin αv was found in the glia cells (L.N.P, arrows). Q-X: Sections of the crushed (R-T.V-X) and contralateral control (Q.U) optic nerves were stained with VTN (Q-T), Intαv (U-X), and DAPI (Q.R.T.U.V.X) at three days after optic nerve crush. Increased expression of vitronectin and integrin αv can be found in the crush line within the optic nerve. In these regions, vitronectin was found primarily in glial cells near the crush sites. Several glial cells (X, arrows) seem to express integrin αv on their cell surface as seen in the high-power field (S.T and W,X from R and V, respectively). The scale bars represent 100 μm in A.B.I.J; 25 μm in C-H.K-P.S.T.W.X; and 50 μm in Q.R.U.V.
larly, increased expression of integrin αv was observed in the optic nerve head three days after the crush injury (Figure 4N). Induction of vitronectin did not co-localize with glia cells marked by DAPI, and seemed likely to be in the extracellular matrix of optic nerve head. The increased expression of integrin αv was primarily located in glial cells, which are distributed either within the septum or around the central retinae artery tract. In the optic nerve proper, faint immunoreactivities of vitronectin and integrin αv were observed in the normal nerve (Figure 4Q,U). After optic nerve crush, the crush sites (arrows in Figure 4R,V) could be clearly identified by the abrupt interruption of neurofilament staining (data not shown) in the optic nerve. Many glial cells at crush sites showed an induction of vitronectin expression (Figure 4S,T). There was also a higher level of vitronectin expression in extracellular matrix. Within the crush line, several cells with an increase of integrin αv expression were also found (Figure 4W,X).

**DISCUSSION**

In a previous study [34], we analyzed the early change in gene expression in the retina after an optic nerve crush by a suppression subtractive hybridization approach. Among the genes detected in the analysis, vitronectin was found in a subtractive retinal cDNA library that contains genes whose expression is upregulated at 24 h after the nerve injury [34]. To verify this observation, we examined whether a rise of vitronectin mRNA and protein could be detected after the nerve injury. Our results demonstrate that there is a 2-fold upregulation of vitronectin mRNA in the retina between one day and three days following optic nerve injury. Moreover, elevated expression of both vitronectin and integrin αv proteins was present in the inner retina, the optic nerve head, and the optic nerve. As the massive death of RGCs does not occur until seven days after injury [23,24], this induction is thus unlikely due to the result of apoptosis of RGCs.

Several lines of evidence have implicated that both extraneuronal factors and intrinsic alterations in aged neurons result in the limited capacity for axonal growth in the adult mammalian RGCs [16,20-22]. It is known that myelin-derived growth inhibitors, such as Nogo [17], MAG [18,19], and OMgp [39]; gliotic scar [15]; and extracellular matrix molecules, like chondroitin sulfate proteoglycans [40-42] and tenascin [43,44] inhibit axonal growth in the adult CNS. Furthermore, adult RGCs show a progressive loss of responsiveness to factors that stimulate axon outgrowth, thus restricting the extent of RGC regeneration after crush injury [21,22]. For example, RGCs from E6 chicks, but not those from E11, extend profuse neurites on laminin [45]; and RGCs from E14 hamsters can regrow into adult tectum under an explant coculture paradigm, but postnatal RGCs cannot innervate tectal targets, even when the target is embryonic [21]. Thus, to facilitate successful regeneration after retinal axonal injury, it is necessary to enhance expression of intrinsic growth-related genes in adult RGC neurons, while neutralizing the extraneuronal inhibitory molecules. Supporting this thought, it has been shown that ectopic expression of GAP43 and CAP 23, two genes involved in the axonal outgrowth, in adult CNS neurons stimulate their regeneration [31].

Vitronectin is present in many different tissues, including brain [1], during development. Vitronectin immunoreactivity has been found in most neuroepithelial cells at E5 in chick retina, and on the nerve fiber and inner plexiform layer at E9 [10]. In adult human, expression of vitronectin is found in the photoreceptor layer and RGC layer of retina and in the septa stroma of optic nerve [6,46]. In our study expression of vitronectin is similarly detected in adult mouse retina, specifically in the RPE, photoreceptor, and RGC (Figure 2C). For expression of vitronectin receptors, it was previously reported that integrin αv is expressed in developing chick retina at least since E6 [11], and on the apical membrane of fetal human RPE and adult primate photoreceptors and RPE [8,47]. We found that, in addition to RPE, the expression of integrin αv in adult mouse retina is present in the RGC layer, inner and outer nuclear layers, the outer plexiform layer, and inner segments of photoreceptors (Figure 2K). Functionally, vitronectin regulates cell differentiation, neuronal survival, and neurite outgrowth in the developing chicken retina [10], and purified E10 chick RGCs grow better on vitronectin than on laminin [13]. These studies implicate vitronectin as an important component in the retinal extracellular matrix for axonal growth. Our data here shows that there is an upregulation of vitronectin in the inner retina, and this may indicate an attempt of RGCs to repair after injury by generating a favorable extracellular environment. The upregulation of integrin αv could also render RGCs responsive to this changing milieu.

An increase in vitronectin and integrin αv is observed in glial cells within the optic nerve head after injury. Changes of extracellular matrix components have been observed following brain injury [48,49], and there is evidence that integrin expression on astrocytes is upregulated in pathological states [50]. Whether this phenomenon represents an overall beneficial or detrimental effect is yet to be fully determined. As different subtypes of integrin expressed on astrocytes affect the permissiveness for neurite outgrowth in a coculture system [51,52], and as vitronectin is known to promote axonal growth [10,12,13], upregulation of integrin αv and vitronectin in the astrocytes within the optic nerve may contribute to RGC axonal regeneration by fostering a growth-promoting environment. On the contrary, another possible scenario after optic nerve injury is that the increase of vitronectin expression in the optic nerve may result from an extravasation of serum vitronectin through damaged vessels at the crush injury. With increased expression of integrin on cell surface, glial cells migrate and proliferate in response to the increased vitronectin, which in turn promotes reactive gliosis and hinders the regeneration of RGC axons in the optic nerve head [53]. In the retina, however, extravasation of plasma vitronectin is less likely to occur, since a local elevation of vitronectin mRNA is detected in the retina, and the injured site lies far away from the retina.

In conclusion, our results show that there is an increase of vitronectin and integrin αv in the retina and the optic nerve after optic nerve injury. Induction of vitronectin and integrin
αν may have an important role in the regeneration of adult mammalian RGCs. Thus, it will be interesting to test whether an exogenously applied stimulus, which elevates the amount of vitronectin in the retinal nerve fiber layer and optic nerve and enhances expression of integrin α in RGC, could facilitate the visual recovery.

ACKNOWLEDGEMENTS
The authors thank L.-S. Kao for helpful comments and critical reading of the manuscript. This paper was supported by grants from Taipei Veterans General Hospital (VGH 91-217), National Science Council (90-2314-B-075-130), and VGH-UST (VTY91-P1-11) of Tsou’s Foundation to A.-G. Wang and M.-J. Fann, and National Health Research Institutes (NHRI-GT-EX89S732C) to M.-J. Fann.

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