GDNF Regulates Chicken Rod Photoreceptor Development and Survival in Reaggregated Histotypic Retinal Spheres

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Purpose. To investigate the role of glial-cell-line-derived neurotrophic factor (GDNF) on proliferation, differentiation, and apoptosis of different retinal cell types—in particular, photoreceptor cells.

Methods. Reaggregated histotypic spheroids, derived from retinal cells of the B6 chicken embryo were used. Under rotation, so-called rosetted spheroids arose by aggregation of dissociated retinal cells, followed by the proliferation, migration, differentiation and programmed cell death of particular cell types. Rosetted spheroids were cultured under serum-reduced conditions, either in the absence or presence of 50 ng/mL GDNF. At appropriate stages, rosetted spheroids were analyzed by using conventional staining and immunolabeling with antibodies against different retinal cell types.

Results. At early stages of culture, the application of GDNF to rosetted spheroids significantly increased and sustained the rate of proliferation. In particular, a de novo production of rod photoreceptors was observed, whereas cone photoreceptors and amacrine, horizontal, ganglion, and Müller cells were not affected. In addition, in GDNF-treated cultures, rod photoreceptors differentiated earlier than in nontreated cultures. In older rosetted spheroids raised in absence of GDNF, rod but not cone photoreceptors underwent apoptosis. By supplementation with GDNF, the percentage of dying rod photoreceptors was dramatically reduced (31%-6% at 8 days in culture, 71%-3% at 10 days in culture). Both the mitogenic and survival promoting effect of GDNF were dose dependent.

Conclusions. The results strongly suggest that GDNF, at least in vitro, affects rod photoreceptors. Depending on the developmental stage, GDNF regulates their proliferation, differentiation, and survival. (Invest Ophthalmol Vis Sci. 2003;44: 2221–2228) DOI:10.1167/iovs.02-0915

GDNF is a distant member of the transforming growth factor (TGF)-β superfamily. GDNF and the related polypeptides neurturin (NRTN), artemin (ARTN), and persephin (PSPN) belong to the GDNF superfamily. GDNF and the related polypeptides neurturin (NRTN), artemin (ARTN), and persephin (PSPN) belong to the GDNF superfamily. GDNF has the highest affinity for GFRα1, whereas NRTN preferentially binds to GFRα2, ARTN to GFRα3, and PSPN to GFRα4.

Originally, GDNF as the first known member of the GFLs, was identified as a neurotrophic factor that prevents dopaminergic neurons from cell death.5 Then it became clear that GDNF can also act as a potent trophic factor for developing enteric, sympathetic, parasympathetic, sensory, and motor neurons.6-17 In addition, GDNF attracts attention because of its possible therapeutic application for the treatment of various neuronal degenerations, such as Parkinson’s, Alzheimer’s, and Hirschsprung’s diseases.18-23 However, as in other parts of the nervous system, the role of GDNF in the embryonic retina is not well understood. Recently, it has been shown that GDNF and its receptors GFRα1 and GFRα2 are expressed in an overlapping and specific pattern in the developing chicken retina.24 GDNF has been found throughout retinogenesis in all retinal cell layers, whereas the expression of GFRα1 and -2 is restricted to particular cell types. GFRα1 has been detected in amacrine and horizontal cells, whereas GFRα2 expression has been observed in amacrine, ganglion, and photoreceptor cells. This spatial expression pattern may explain the results of previous studies that have shown GDNF can act as a trophic factor for both retinal ganglion cells and photoreceptors and therefore serve as a useful therapeutic tool to restore deficient cell types caused by ophthalmic disorders.25-30 Furthermore, treatment of photoreceptor-enriched rat monolayer cultures with GDNF results in an increase of photoreceptor precursors within the first hours in culture, and delays the onset of programmed cell death at a later stage.31 Although several studies have shown an effect of GDNF on photoreceptors, it is not clear whether GDNF acts on all photoreceptors in a general manner or is restricted to either rod or cone photoreceptors. Likewise, little is known about the effect of GDNF on the other retinal cell types, such as bipolar, amacrine, horizontal, and Müller cells. It is now accepted that a series of soluble factors can influence the fate of retinal precursor cells (RPCs).32 These factors are mostly derived from retinal cells or adjacent tissue in close approximation to uncommitted RPCs. In this way, RPCs receive information from the environment (extrinsic signals), and this in turn determines the fate of specific cell types. However, in addition to extrinsic factors it has been postulated that intrinsic properties of cells also contribute to cell fate decisions. This means that the three-dimensional environment is essential for proper retinal development.

In our experiments we used reaggregated organotypic retinal spheres, called rosetted spheroids, with the advantage that they imitate a de novo retinal development in three-dimensional in vitro conditions and can be easily manipulated by the addition of GDNF. Under rotation conditions, rosetted spheroids arise through aggregation of dispersed retinal cells of the chicken embryo, followed by the proliferation, migration, differentiation, and programmed cell death of particular cell types. After 2 weeks in culture, mature rosetted spheroids represent composites of morphologic structures homologous to all retinal layers (Fig. 1F; for detail see Refs. 33-35). First,
rosetted areas consist of rosettes, their internal lumen lined with photoreceptors, thus corresponding to the outer nuclear layer (ONL) of a normal retina. Each rosette is surrounded by a circular outer plexiform layer (OPL), followed by sections of an inner nuclear layer (INL). Second, inner plexiform layer (IPL)-like areas resemble an in vivo IPL, because they are mainly cell-free areas consisting of fibers composed of bipolar, ganglion, and amacrine cells. Within these circular IPLs, only a few displaced ganglion cells were detectable. Moreover, there were nonorganized areas consisting of different retinal cell types and their corresponding fibers.

In the current study, we applied GDNF to rosetted spheroid cultures to investigate its role in proliferation, differentiation, and survival during development of the chicken retina in vitro. GDNF increased and sustained proliferation of retinal cells, promoted the onset of differentiation of rod photoreceptors, increased their number, and effectively prevented programmed cell death. In contrast, it did not induce increases the number of cone photoreceptors or ganglion, amacrine, horizontal, or Müller cells.

**METHODS**

**Tissue Culture**

To produce rosetted spheroids, central parts of the retinas of 6-day-old chicken embryos (white leghorn) were isolated and collected in F12 medium on ice. The tissues were fully dissociated by tryptic digestion in Hanks’ balanced salt solution (HBSS) containing 0.05 mg/mL trypsin (Worthington Biochemicals/Cell Systems, Remagen, Germany) for 5 minutes at 37°C. After two washes in F12 medium, including tissue sedimentation, the remaining cell clusters were mechanically dissociated in F12 medium containing 0.5 mg/mL DNase I (Worthington Biochemicals/Cell Systems) by 30 to 40 gentle strokes with a round-bored Pasteur pipette. For the generation of rosetted spheroids, 2×10⁶ cells/mL were cultivated, either in the presence or absence of 50 ng/mL rat GDNF (Sigma, Deisenhofen, Germany) in 35-mm dishes containing 2 ml aggregation medium (DMEM, 2% FCS, 1% L-glutamine, and 0.15% penicillin/streptomycin, all from Gibco, Berlin, Germany) on a gyratory shaker in a Heraeus incubator (37°C.)
BrdU was added 16 hours before the spheroids were harvested and for immunostaining.

Cryosections

For cryosections, rosetted spheroids were harvested at appropriate stages and fixed in 4% formaldehyde (Merck, Darmstadt, Germany) for 30 minutes at room temperature. After the fixative was removed and two washes performed in PBS, rosetted spheroids were soaked in 25% sucrose (Merck, Darmstadt, Germany) and stored at 4°C. Frozen sections of 10 to 12 μm thickness were cut on a cryostat (Microm, Heidelberg, Germany), mounted on gelatin-coated slides, and stored for immunostaining at −20°C. For BrdU-incorporation experiments, BrdU was added 16 hours before the spheroids were harvested and fixed as just described.

Primary Antibodies

The polyclonal antiserum CERN 901 was raised against purified chicken rhodopsin, and the antiserum CERN 906 was raised against purified chicken red and green pigments. The CERN antibodies (a generous gift of Willem DeGrip, University of Nijmegen, Nijmegen, The Netherlands) were used at a dilution of 1:2000 in PBS (0.1% Triton-X-100) and were incubated for 1 hour at room temperature. BrdU antibodies (Sigma, Deisenhofen, Germany) were used 1:1000 whereas Pax-6 hybridoma supernatant (specific for amacrine, ganglion and horizontal cells in differentiated retinal cells of the chick57–59; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) was added undiluted.

Staining Procedures

For immunostaining, sections were dried at 37°C and preincubated in blocking solution (3% BSA, 0.1% Triton-X-100 in PBS) for 30 minutes at room temperature. The tissues were then incubated with the appropriate primary antibodies, followed by three washes in PBS. For detection of the primary antibodies, either goat-anti-rabbit-Cy2 or donkey anti-mouse-conjugated-Cy3 secondary antibodies were applied for 1 hour at a dilution of 1:100 in PBS. Between the last two washes, cell nuclei were stained with DAPI (0.1 mg/mL 4’,6-diamidino-2-phenylindol-dihydrochloride in PBS). Finally, sections were dried and embedded in Kaiser's glycerin gelatin (Merck, Darmstadt, Germany). For double-labeling experiments, sections were stained with TUNEL red (according to the manufacturer's instructions; Roche Molecular Biochemicals, Mannheim, Germany), followed by immunostaining with the primary antibodies described. For the detection of BrdU, we used the same procedures as just described, with the exception that sections were treated first with 4 N HCl for 5 minutes before the BSA-containing blocking solution was added.

Cell Counting and Statistical Analysis

To determine the number of immunopositive and TUNEL-positive cells, frozen sections (each containing 30–40 spheroids) were stained with DAPI and the corresponding antibodies or staining reagents, respectively. The percentage of immunolabeled cells per cryosection of a single spheroid section. At least nine cryosections of different spheroids were analyzed (nine spheroid section. At least nine cryosections of different spheroids were analyzed (nine spheroid sections and stained with DAPI). The corresponding antibodies or staining reagents, respectively. The percentage of immunolabeled cells was calculated in relation to DAPI-positive cells of the same spheroid section. At least nine cryosections of different spheroids derived from two individual experiments were analyzed (nine spheroids investigated). This corresponds to counting of 4000 to 5000 cells per stage and staining. Data are presented as the mean ± SD and compared by a two-tailed, paired Student's t-test. Note that the standard deviation represents the differences between the calculation of individual spheroids.

Microscopy and Photography

Photomicrographs of sections were taken with a microscope (Axioskop; Carl Zeiss, Jena, Germany) combined with a charge-coupled device, three-color (CCD-3) digital camera (Intas, Göttingen, Germany) and processed on computer (focus imager model 4000; Intas, Photopoint 5.0; Adobe, Mountain View, CA; and PowerPoint, Microsoft, Redmond, WA).

RESULTS

Effect of GDNF on Proliferation of Retinal Cells at Early Stages of Spheroid Development

Exogenous application of GDNF stimulated the proliferation of retinal cells in rosetted spheroids (Figs. 1A–D). The quantitative data obtained at appropriate stages are shown in Figure 1E. GDNF-treated spheroids showed a significantly increased BrdU uptake during the first 6 days in culture. After 2 days in culture, the number of proliferating cells was still very high, 17% more BrdU-positive cells were detected in GDNF-treated spheroids (Figs. 1A, 1B, 1E). After 4 days in culture, the rate had significantly dropped, but the effect became much more pronounced (Figs. 1C, 1D, 1E); now, 257% more BrdU-positive cells were detectable in the presence of GDNF. At day 6, GDNF increased the rate of proliferation up to 185%. At later stages (days 8–10) proliferation had become very low and did not differ significantly when compared with control spheroids (Fig. 1E).

Effect of GDNF on the Number of Rod Photoreceptors and the Onset of Differentiation

Analyzing the course of spheroid development, a continuously increasing number of rods was revealed within the first 8 days of culture in both treated and nontreated spheroids (Fig. 2A, cf. Figs. 4A, 4B). However, at all stages the number of rods was significantly increased in the presence of GDNF (Fig. 2A). Remarkably, from days 8 to 10, the number of rod photoreceptors in nontreated spheroids decreased by 41%. A striking opposite effect was detected in GDNF-treated spheroids. Here, the number of rods was further increased from days 8 to 10, resulting in a dramatic increase of rod photoreceptors, when compared with control spheroids at stage 10 (Fig. 2A; green cells in Figs. 4A, 4B). GDNF affected rod photoreceptor differentiation at a very early stage of culture. In GDNF-treated spheroids, a small number of rods was detectable as early as 2 days (Figs. 3A, 3B), whereas at this time in nontreated spheroids, rod photoreceptors were not yet detectable (Figs. 3C, 3D). It is remarkable that these early rods achieved an advanced state of maturation, as indicated by opsins expression on their cell surfaces, extending into processes (Fig. 3A, inset).

Protection of Rod Photoreceptors from Programmed Cell Death

In the presence of GDNF, the number of rod photoreceptors did not decrease from days 8 to 10 (cf. Fig. 2A), as observed in the absence of GDNF, indicating a specific effect of GDNF on survival of rods. Therefore, we used either a rod/TUNEL or a cone/TUNEL double-labeling technique in combination with DAPI staining to visualize apoptotic photoreceptors. Rod (Figs. 4A, 4B) and cone (Figs. 4C, 4D) photoreceptors appear green, apoptotic cells red, and apoptotic photoreceptors yellow in Figure 4, because opsins and defragmented DNA are colocalized in the same cell. Cone photoreceptors did not undergo apoptosis, regardless of whether spheroids were treated with or without GDNF (Figs. 4C, 4D). The situation was dramatically different for rod photoreceptors (Figs. 4A, 4B). After 10 days in culture in the absence of GDNF, there was a significant number of apoptotic rods (Fig. 4A, yellow), which, remarkably, in GDNF-treated spheroids was dramatically decreased (Fig. 4B).
Rod Photoreceptors

Concentration Dependent Effect on Proliferation of Various Cell Types

Effect of GDNF on Proliferation of Various Cell Types

To investigate whether the proliferative effect of GDNF is exclusively restricted to the rod cell lineage, we used several specific antibodies to quantify the number of cone photoreceptors, amacrine cells, retinal ganglion cells, horizontal cells, and Müller cells in treated and nontreated spheroids. In contrast to rod photoreceptors, no significant elevation of cone photoreceptors was achieved in GDNF-treated spheroids (Figs. 2A, 2B), and the temporal expression of cone photoreceptors resembled that of rod photoreceptors; there was no decrease in cones after day 8. Apart from differences in number of cells, the local distribution of rod and cone photoreceptors was identical within treated and nontreated spheroids (compare green cells in Figs. 4A, 4B with Figs. 4C, 4D). Most of the photoreceptors were localized in nonorganized areas (arrows); only a smaller number occurred in rosettes (stars).

In comparison with DAPI staining, most Pax6-positive cells were detected in INLs surrounding a circular IPL (Figs. 5A, 5C), whereas only a few Pax6-positive cells were detectable within the IPL (Figs. 5A, 5C, arrows) and nonorganized areas (Figs. 5A, 5C, arrowheads). The number of Pax6-positive cells was nearly identical in nontreated and treated spheroids. At days 8 and 10 of culture, we found no quantitative differences in the number of Pax6-positive cells between treated and nontreated spheroids (Fig. 5E). Similarly, based on glutamine synthetase staining, the number of Müller cells did not change after application of GDNF (data not shown).

Concentration Dependent Effect on Proliferation of Retinal Cells and on Survival of Rod Photoreceptors

To examine whether the observed increase in proliferation and survival of rod photoreceptors was specifically induced by GDNF, spheroids were grown in the presence of 0, 0.5, 5, 25, 50, and 100 ng/mL GDNF. As shown in Figure 4F, both the increase in the rate of proliferating cells at stage 4 (diamonds) and the decrease in the number of apoptotic rod photoreceptors at stage 10 (squares) followed a dose-response curve characteristic of growth factors and neurotrophins. In both cases, the effect on proliferation and on survival was enhanced with increasing concentrations of GDNF, reaching saturation at approximately 50 ng/mL. The EC50 of GDNF for proliferation and for survival of rods both amounted to 2.5 ng/mL.
DISCUSSION

This study demonstrates for the first time that GDNF has multiple effects on rod photoreceptors during in vitro development of the chicken retina. Depending on the developmental stage, GDNF affected proliferation, onset of differentiation, and survival of rod photoreceptors.

Effect of GDNF on Proliferation and Onset of Rod Photoreceptor Differentiation

In a series of previously published reports, a proliferative effect of GDNF has been described in nonretinal tissue. In particular, in the enteric nervous system, it has been shown that GDNF is essential for the proliferation of enteric precursor cells.11–13,15,40,41 Similarly, GDNF has been found to stimulate proliferation during development of kidneys in vivo and in vitro.42–44 Beyond this, a regulatory mitogenic effect has been demonstrated for rat glioma cells by adding exogenous GDNF45 or by using antisense oligonucleotides for the suppression of endogenous GDNF.46 A proliferative effect of GDNF has been established in photoreceptor-enriched rat monolayer cultures.31 Our data profoundly extend these findings. We showed a specific function of GDNF on rods within a histotypic three-dimensional tissue context consisting of all retinal cell types. GDNF not only increased proliferation but also sustained the phase of proliferation up to day 6 in culture. Moreover, GDNF increased proliferation in a dose-dependent fashion, reflecting the specificity of GDNF as a mitogenic factor. The expanded period of proliferation resulted mainly in an increased number of rods, whereas other retinal cell types like cones and Müller, amacrine, ganglion, and horizontal cells did not respond to GDNF. This indicates that rod photoreceptor precursors are positively affected by GDNF, because at a very early stage of culture (up to day 6), the number of differentiated rod photoreceptors was always higher than in cultures treated with GDNF.31 Cone photoreceptors in both non-GDNF-treated spheroids (D) showed many fewer TUNEL-positive cells. Note that, in addition to rods, other retinal cell types also underwent apoptosis (red). The percentage of apoptotic rods was quantified by the rod/TUNEL double-labeling technique in relation to the total number of rods per spheroid section. At days 8 and 10 in culture, the ratio of TUNEL-positive rods was dramatically reduced in the presence of GDNF. (F) Rossetted spheroids were grown in the presence of 0, 0.5, 5, 25, 50, and 100 ng/mL GDNF and were harvested either after 4 days (for calculation of BrdU-positive cells) or after 10 days (for quantification of apoptotic rods) in culture. GDNF acted in a dose-dependent manner on proliferation (EC50 = 2.5 ng/mL) and survival of rod photoreceptors (EC50 = 2.5 ng/mL). Each data point represents the mean ± SD of multiple spheroids sections (n = 9).

FIGURE 4. GDNF treatment promoted survival of rod photoreceptors in rosetted spheroids. Cryosections of 10-day-old rosetted spheroids were double stained, either with TUNEL (red) and the rod-specific antibody CERN 901 (A, B, green), or with TUNEL and the cone-specific antibody CERN 906 (C, D, green). Most of the photoreceptors were localized in nonorganized areas (arrows), with only a small number detected in rosettes (+). (A) In nontreated spheroids, the number of apoptotic rods (yellow, colocalization of TUNEL and rod staining) was much higher in cultures treated with GDNF (B). Cone photoreceptors in both non- (C) and GDNF-treated spheroids (D) showed many fewer TUNEL-positive cells. Note that, in addition to rods, other retinal cell types also underwent apoptosis (red). The percentage of apoptotic rods was quantified by the rod/TUNEL double-labeling technique in relation to the total number of rods per spheroid section. At days 8 and 10 in culture, the ratio of TUNEL-positive rods was dramatically reduced in the presence of GDNF. (F) Rossetted spheroids were grown in the presence of 0, 0.5, 5, 25, 50, and 100 ng/mL GDNF and were harvested either after 4 days (for calculation of BrdU-positive cells) or after 10 days (for quantification of apoptotic rods) in culture. GDNF acted in a dose-dependent manner on proliferation (EC50 = 2.5 ng/mL) and survival of rod photoreceptors (EC50 = 2.5 ng/mL). Each data point represents the mean ± SD of multiple spheroids sections (n = 9).
(CNTF) also increased the number of rod precursors in chick retina monolayer cultures, whereas cones were unaffected. We have shown that the number of rod photoreceptors depends on microenvironmental changes—in particular, rods developed in spatial proximity to preexisting cones. Their development, in turn, was autonomous. These data support the idea that the development of rods in contrast to cones is dependent on various extrinsic signals, one of these factors presumably being GDNF. An alternative, yet at the most only partial, explanation for the missing effect of GDNF on cone photoreceptors could be related to the age of the retinal tissue (embryonic day 6) that is used for the production of rosetted spheroids. In contrast to rods which appeared late during retinogenesis, a proportion of cones were already postmitotic by embryonic day 6. Therefore, such cone precursors may be incapable of responding to GDNF at that point. Further investigations with rosetted spheroids derived from retinal tissues younger than embryonic day 6 are needed.

Furthermore, our study showed that GDNF promotes the onset of rhodopsin expression in spheroids within 2 days. Even though they appeared early in spheroid development, these rods expressed high levels of opsin, therefore representing an advanced stage of photoreceptor differentiation. This means that the action of GDNF was restricted to rod photoreceptors, and it not only induced the proliferation of this cell type, it also accelerated its differentiation.

Prevention of Apoptosis in Rod Photoreceptors at Late Stages of Retinal Development In Vitro

Programmed cell death is a typical feature of retinal development, occurring in different retinal cell types of the chicken embryo but not in photoreceptors. To induce cell death of photoreceptors, we produced spheroids under reduced serum conditions. Although the concentration of serum was low, spheroids showed an intact and well-organized morphology. This means that apoptosis in spheroids occurred, but took place in a proper cellular environment and was not induced by the necrotic processes. Therefore, the use of spheroids represents a suitable culture system to investigate the survival effect of GDNF on different retinal cell types, particularly rod photoreceptors. A survival-promoting effect of GDNF has been reported in certain cell types of the retina. Photoreceptors and ganglion cells seem to be especially sensitive to GDNF. In this context, it has been shown that GDNF increased the survival time of rod outer segments in vitro. Moreover, sub-retinal injection of GDNF into the eyes of rd/rd mice prevents photoreceptors from cell death. In organ cultures of rd mice, GDNF alone is unable to prevent photoreceptor cell death, but in combination with CNTF, a partial rescue of photoreceptors has been observed. Therefore, the authors speculated that GDNF and other growth factors act synergistically rather than individually. In contrast to this, it has been shown that in rat...
retinal cultures GDNF alone is able to increase the survival of rods, but in combination with docosahexaenoic acid, this effect is dramatically enhanced. Nevertheless, our study clearly showed that GDNF can effectively protect rod photoreceptors from cell death without addition of any substance. In the presence of GDNF only 2.8% of all rods were apoptotic, whereas in untreated cultures 71% of rods underwent programmed cell death after 10 days in culture. This means that GDNF prevented cell death of rod photoreceptors by a factor of 25. Therefore, GDNF could become a potential therapeutic tool for the treatment of a series of retinal degenerations that are primarily characterized by the loss of rod photoreceptors.

Moreover, we found that the survival-promoting effect of GDNF acted in a dose-dependent manner, reflecting the specific action of GDNF on the survival of rods. Our results also showed that cone photoreceptors did not undergo apoptosis under serum-reduced conditions. This strongly suggests that the survival of rod and cone photoreceptors is regulated by two independent mechanisms. A GDNF-dependent mechanism rescues rods from programmed cell death, whereas the survival of cone photoreceptors is GDNF independent, or probably does not require any additional signal for survival. In contrast to cones, the populations of retinal ganglion and amacrine cells undergo programmed cell death during spheroid development, and their loss is not counteracted by GDNF. This observation is surprising, because GFRα1 is expressed in ganglion cells and GFRα2 is expressed in both amacrine and ganglion cells in the chicken retina. Therefore, we postulate that GFRα1/GDNF signaling is not responsible for survival of these retinal cell types, or alternatively, additional factors are needed to rescue them from programmed cell death. In striking contrast, the survival of rod photoreceptors was positively affected, but GFRα1, which preferentially binds GDNF, is not expressed in the ONL. Instead, only GFRα2 is exclusively expressed in the photoreceptor layer. This indicates that at least in the chicken retina, survival of rod photoreceptors is probably regulated through the interaction of GDNF and GFRα2, but not by the GFRα1/GDNF signaling pathway.

In conclusion, in our study GDNF showed different functions during retinal development in vitro: It acted as a mitogenic factor for rod photoreceptors at early stages; influenced the onset of differentiation of rod photoreceptors; and supported photoreceptor survival at later stages. Moreover, in contrast to rods, a de novo production of other retinal cell types was not stimulated by GDNF. For further understanding of the role of GDNF in rod photoreceptor regulation, we have begun to investigate the temporal and spatial expression of GDNF and its receptors during retinal development.

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References


