Title
GDF11 Controls the Timing of Progenitor Cell Competence in Developing Retina

Permalink
https://escholarship.org/uc/item/42r9d2rk

Journal
Science, 308(5730)

ISSN
0036-8075

Authors
Kim, Joon
Wu, Hsiao-Huei
Lander, Arthur D
et al.

Publication Date
2005-06-24

DOI
10.1126/science.1110175

Supplemental Material
https://escholarship.org/uc/item/42r9d2rk#supplemental

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of PKR2-positive cells in the rostral portion of the RMS and OV of PK2−/− mice (fig. S12), also shown by PSA-NCAM immunostaining (Fig. 3E). Analysis of PKR2 mRNA expression in PK2−/− mice showed an overall decrease in neuronal progenitors migrating away from the OV into the GL and PGL (fig. S12). The compaction of PKR2-positive cells in the OV indicates that, in the absence of PK2 signaling, channeled neuronal progenitors are either not detached properly or disoriented about the direction of migration. To evaluate whether PK2 is a genuine chemoattractant for SVZ neuronal progenitors, we performed SVZa explants coculture assay with the GL of the OB, where PK2 is primarily expressed (Fig. 1, D and E). Cell migration was directed toward the GL tissue from WT OB, whereas the corresponding tissue from PK2−/− OB exhibited no chemotaxis activity (proximal/distal ratio: 1.87 ± 0.31 versus 0.99 ± 0.06, WT versus PK2−/−, respectively; n = 6 explants, P < 0.05) (fig. S13). Taken together, these results indicate that the migration of neuronal progenitors mediated by PK2 signaling is essential for the normal development and maintenance of the OB.

Thus, PK2 serves as a chemoattractant for SVZ-derived neuronal progenitors, and the establishment of normal OB architecture requires PK2 signaling. Together with other signals (12, 13, 15), PK2 appears to guide the migration of neuronal progenitors from the SVZ through the RMS to their final layers in the OB. The similar response of PKR1 and PKR2 to PK2 (22) implies that these receptors may mediate a redundant role for OB development. As with endothelin-3 signaling for the migration of enteric neurons (33) and orphan receptor GPR56 in the regional development of the cerebral cortex (34), our results further indicate that G protein–coupled receptors may be crucial for the establishment of the layered structures in the nervous system.

References and Notes
1. J. Altman, J. Comp. Neurol. 137, 433 (1969).
2. F. H. Gage, Science 287, 1433 (2000).
3. M. S. Kaplan, J. W. Hinds, Science 197, 1092 (1977).
4. S. A. Bayer, Exp. Brain Res. 50, 329 (1983).
5. V. Penceka, K. D. Bingaman, L. J. Freedman, M. B. Luksin, Exp. Neurol. 172, 1 (2001).
6. M. B. Luksin, Neuron 11, 173 (1993).
7. C. Lois, A. Alvarez-Buylla, Science 264, 1145 (1994).
8. B. H. Wichterle, J. M. Garcia-Verdugo, A. Alvarez-Buylla, Neuron 18, 779 (1997).
9. H. Tomaszewicz et al., Neuron 11, 1163 (1993).
10. H. Cremer et al., Nature 367, 455 (1994).
11. K. Ono, H. Tomaszewicz, T. Magnuson, U. Rutishauser, Neuron 13, 595 (1994).
12. I. Hack, M. Bancila, K. Loulier, P. Carroll, H. Cremer, Nat. Neurosci. 5, 939 (2002).
13. A. Agathangelou, A. de Chevigny, M. Schachner, P. M. Lledo, Nat. Neurosci. 7, 347 (2004).
14. H. Hu, U. Rutishauser, Neuron 16, 933 (1996).
15. W. Wu et al., Nature 400, 331 (1999).
16. G. Liu, Y. Rao, J. Neurosci. 23, 6651 (2003).
17. M. Li, C. M. Bullock, D. J. Knauer, F. J. Ehler, Q. Y. Zhou, Mol. Pharmacol. 59, 692 (2001).
18. M. Y. Cheng et al., Nature 417, 405 (2002).
19. J. LeCouter et al., Proc. Natl. Acad. Sci. U.S.A. 100, 2685 (2003).
20. C. Mollay et al., Eur. J. Pharmacol. 374, 189 (1999).
21. L. Negri et al., Br. J. Pharmacol. 137, 1147 (2002).
22. D. C. Lin et al., J. Biol. Chem. 277, 19276 (2002).
23. T. Soga et al., Biochim. Biophys. Acta 1579, 173 (2002).
24. K. L. Ng et al., unpublished data.
25. K. Kishi et al., Arch. Histol. Cytol. 53, 219 (1990).
26. H. Zhang, L. Vutsikis, M. S. Pepper, J. Z. Kiss, J. Cell Biol. 143, 1375 (2000).
27. T. D. Palmer, A. R. Willhoite, F. H. Gage, J. Comp. Neurol. 425, 479 (2000).
28. J. LeCouter et al., Nature 412, 877 (2001).
29. C. M. Bullock, J. D. Li, Q. Y. Zhou, Mol. Pharmacol. 65, 582 (2004).
30. M. Ward, C. McCann, M. DeWulf, J. Y. Wu, Y. Rao, J. Neurosci. 23, 5170 (2003).
31. J. D. Li et al., unpublished data.
32. R. Betarbet, T. Zigova, R. A. Bakay, M. B. Luksin, Int. J. Dev. Neurosci. 14, 921 (1996).
33. A. G. Baynash et al., Cell 79, 1277 (1994).
34. X. Piao et al., Science 303, 2033 (2004).
35. We thank H. van Praag, O. Steward, and C. Zhang for discussions; C. Tu and H. Shen for technical assistance; and the laboratories of C. Cotman and F. Laferla for access to equipment. Supported by a University of California Discovery grant. K.N. is a recipient of a UCI Medical Scientist Training Program training grant.

Supporting Online Material
www.sciencemag.org/cgi/content/full/308/5730/1923/ DC1
Materials and Methods
Figs. S1 to S3
References and Notes
10 March 2005; accepted 2 May 2005
10.1126/science.11212103

GDF11 Controls the Timing of Progenitor Cell Competence in Developing Retina
Joon Kim,1,2 Hsiao-Huei Wu,1,2,* Arthur D. Lander,2,3 Karen M. Lyons,4 Martin M. Matzuk,5 Anne L. Calof1,2†

The orderly generation of cell types in the developing retina is thought to be regulated by changes in the competence of multipotential progenitors. Here, we show that a secreted factor, growth and differentiation factor 11 (GDF11), controls the numbers of retinal ganglion cells (RGCs), as well as amacrine and photoreceptor cells, that form during development. GDF11 does not affect proliferation of progenitors—a major mode of GDF11 action in other tissues—but instead controls duration of expression of Math5, a gene that confers competence for RGC genesis, in progenitor cells. Thus, GDF11 governs the temporal windows during which multipotent progenitors retain competence to produce distinct neural progeny.

The vertebrate neural retina comprises seven neural cell types, all derived from one population of multipotential progenitors (1, 2). Retinal cell types do not arise synchronously but are generated in a stereotyped sequence (3, 4). In vitro results imply that retinal progenitors at different stages differ in their competence to produce distinct cell types (5–7).

Such changes in potential are likely dictated by changes in expression of the transcription factors encoded by proneural genes (8, 9), but mechanisms of proneural gene regulation are poorly understood. An important role for cell-cell signaling is suggested by the fact that production of at least two retinal cell types, retinal ganglion cells (RGCs) and amacrine cells, can increase to compensate for losses of mature cells in either population (10, 11). This process has been postulated to be mediated by a feedback signal produced by mature cells (12), but the identity of the signal(s) is unknown.

GDF11, a member of the transforming growth factor-β superfamily of secreted signaling molecules, is expressed in several regions of a developing nervous system, including the retina (13). In olfactory epithelium (OE), GDF11 negatively regulates neuron number by causing cell-cycle arrest of the progenitor cells that give rise to olfactory receptor neurons (ORNs) (14). Here, we demonstrate that GDF11 is also a negative regulator of neuron number in neural retina, but through a completely different mechanism: GDF11 controls
the period during which retinal progenitor cells are competent to produce certain progeny, thus governing the relative numbers of neural cell types that arise.

In mouse retina, Gdf11 expression begins about embryonic day (E) 12.5, when RGCs begin to differentiate (Fig. 1A). Gdf11 mRNA is observed throughout the retina, including the neuroblastic layer (NBL), until at least the first postnatal day (P0), although by E15.5, expression is highest in the developing ganglion cell layer (GCL). Expression of follistatin (Fst), which encodes a secreted GDF11 antagonist, is highest in the nascent GCL (which encodes a secreted GDF11 antagonist) but also evident in the NBL and presumptive amacrine cells. Putative receptors for GDF11 (14, 16–18) are also expressed in appropriate patterns in the neural retina from E12.5 to 13.5 onward (fig. S1).

To investigate the role of Gdf11 in retinal development, we examined mice homozygous for the null allele Gdf11 (14). Gdf11 mice show obvious changes as early as E14.5, when closure of the optic fissure is incomplete (fig. S2). By E17.5, the presumptive GCL of mutant embryos has an abnormally high cell density, and the inner plexiform layer (IPL), well demarcated in wild-type littermates, is not observed (Fig. 1B). Increased cell density in the mutant GCL is accompanied by widening of the cell layer expressing Brn3b (Gdf11tm2/m2, 49.5 ± 3.3 μm (SD); wild-type, 38.5 ± 0.4 μm (SD)), which encodes a POU-domain transcription factor specific for differentiated RGCs (19, 20). By P0, the latest time at which the mutant is viable, Gdf11tm2/m2 GCLs contain ~50% more cells than wild types (Fig. 1C). The excess RGCs that form in Gdf11tm2/m2 animals appear to differentiate normally, extending axons through the optic chiasm and tracts, which also appear abnormally thick (Fig. 1D). By neurofilament immunohistochemistry, we estimate a 37% increase in the cross-sectional areas of optic nerves in Gdf11tm2/m2 animals (Fig. 1E and fig. S2).

These changes, observed in all mutant mice examined (>32), imply that Gdf11 is a negative regulator of RGC genesis. In this respect, the changes in Gdf11tm2/m2 retinas recall those in OE, in which Gdf11tm1/jtm2 mice also have excess differentiated ORNs (14). However, unlike the situation in OE (14), Gdf11tm2/m2 retinas display no increase in overall thickness, nor are the distribution or number of proliferating cells significantly altered (fig. S3). These observations suggest that the mechanism by which Gdf11 regulates neurogenesis in the retina differs from that in the OE.

Because Fst is known to antagonize GDF11 function in vivo and in vitro (14, 15), we also examined Fsttm2/jtm2 and Gdf11tm2/m2:Fsttm2/jtm2 retinas. Fsttm2/jtm2 retinas showed a 26% reduction in the number of cells in the GCL and a large decrease in thickness of the Brn3b+ cell layer (Fig. 2, A and B), which indicates that Fst is a positive regulator of RGC development.

Gdf11tm2/m2:Fsttm2/jtm2 showed an expanded Brn3b+ GCL, comparable to that observed in Gdf11tm2/m2 retinas, consistent with the primary role of Fst as an inhibitor of GDF11 (Fig. 2B). Just as in Gdf11tm2/m2 retinas, the level and pattern of progenitor cell proliferation was unaltered in Fsttm2/jtm2 retinas (fig. S3). The fact that cell proliferation is normal in Gdf11tm2/m2 and Fsttm2/jtm2 retinas suggests that the size of the progenitor pool is not regulated by GDF11. Moreover, expression of several genes involved in early eye specification, patterning, and expansion is also normal in Gdf11tm2/m2 mice (fig. S4).

During development, RGCs are born at the outer margin of the neural retina and migrate inward to the GCL during a defined period (21). Detailed examination of Gdf11tm2/m2 and Gdf11tm2/m2:Fsttm2/jtm2 retinas at E17.5 revealed that the NBL of these mutants contains three times as many Brn3b+ cells (migrating RGCs) as do wild types (Fig. 2B, insets, and fig. S5). This suggested that, in Gdf11tm2/m2 retinas, RGC production may be prolonged beyond its normal period. To test this, we performed birthdating experiments. The results, shown in Fig. 2C, show an abnormally large number of bromodeoxyuridine-positive (Brdu+) cells in the GCL of Gdf11tm2/m2 animals pulsed with Brdu from E15.5 to E17.5. Conversely, Brdu+ cells in the GCL of Fsttm2/jtm2 animals pulsed over this same time course were strongly decreased in number, as expected if Fst acts to inhibit GDF11. These differences were not seen when pulse labeling was done at earlier ages (Fig. 2E). Thus, although onset of RGC production appears unaffected by loss of Gdf11 or Fst, its down-regulation is delayed in Gdf11tm2/m2 retinas and accelerated in Fsttm2/jtm2 (Fig. 2F).

A lengthened period of RGC production likely explains why Gdf11tm2/m2 retinas accumulate abnormally large numbers of RGCs.

To determine whether Gdf11 regulates production of other retinal cell types, we examined rod photoreceptors and amacrine cells, two cell types whose peak periods of differentiation follow that of RGCs. Cxvl, a marker for early photoreceptors, is normally up-regulated around birth when rod photoreceptor production peaks, and expands to cover much of the NBL (22). In Gdf11tm2/m2 retinas, up-regulation and expansion of Cxvl expression are not observed (Fig. 3A). Amacrine cells may be visualized by expression of syntaxin (23), as well as Pax6 and Proxl (24, 25). In the amacrine cell layer of Gdf11tm2/m2 retinas, expression of all three markers was reduced.
Gdf11tm2/tm2 decreased, in whereas amacrine and rod production are retinal cell types. GDF11 is an important regulator of all three 3B). These findings support the idea that Gdf11petence, GDF11 directly controls progenitor cell com-
tence to produce later-born cell types. If changes by which progenitor cells lose com-
petence to produce RGCs and acquire compe-
tence states.

The finding that RGC genesis is increased, whereas amacrine and rod production are decreased, in Gdf11 nulls led us to hypothesize that Gdf11 regulates induction of cell-intrinsic changes by which progenitor cells lose competence to produce RGCs and acquire competence to produce later-born cell types. If GDF11 directly controls progenitor cell competence, Gdf11 mutants might exhibit changes in expression of factors that determine competence states. Math5 is among the first such factors expressed during retinal neurogene-
sis and is required for competence to pro-
duce RGCs (27–29). Math5 expression is ini-
tiated normally in Gdf11tm2/2tm2 retina, but mutants maintain high levels of expression in the NBL for an abnormally long period. Normally, Math5 expression is down-
regulated in central NBL by E16.5 and is essen-
tially absent by E18.0, in Gdf11tm2/2tm2 retinas, however, Math5 expression is still evident at these ages (Fig. 4A). Conversely, down-regulation of Math5 expression occurs prematurely in Fst−/− retinas (Fig. 4B) and is accelerated when retinal explants are cultured in GDF11 (Fig. 4C). The prolonged period of Math5 expression in Gdf11tm2/2tm2 retina corresponds to the period of prolonged RGC genesis (Fig. 2).

The alteration in the period of Math5 expression in Gdf11tm2/2tm2 retina is accompa-
nied by a shift in onset of expression of two other proneural genes, Mash1 and NeuroD, which are involved in the development of bipolar and amacrine cells (30, 31). In Gdf11tm2/2tm2 embryo, expression of both genes is barely detectable at E14.5, when substantial levels are seen in wild types (Fig. 4D). Conversely, Mash1 expression occurs prematurely in Fst−/− retinas, at E13.5, when wild-type littermates express only low levels of Mash1 (Fig. 4E). By E17.5, both Mash1

![Fig. 2. The period of RGC genesis is altered in Gdf11tm2/2tm2 and Fst−/− retinas.](image)

![Fig. 3. GDF11 regulates development of photoreceptors and amacrine cells.](image)

www.sciencemag.org  SCIENCE  VOL 308  24 JUNE 2005  1929
and NeuroD expression recover to normal levels in Gdf11tm2/tm2 retinas (Fig. 4D), which suggests that progenitor cells can acquire competence to produce later-born cell types even though Math5 expression (and RGC genesis) remain elevated. Altogether, these observations suggest that GDF11 regulates the timing of progenitor competence by controlling the expression of genes crucial for progenitor cell fate determination.

Does GDF11 regulate generation of all retinal cell types, or only some? Because Gdf11tm2/tm2 animals die at birth (14), this question cannot yet be answered with certainty. Expression of Lim1, a horizontal cell-specific transcription factor (32), appears to be normal in Gdf11tm2/tm2 retinas (fig. S6), although changes in expression of a number of other regulatory genes expressed by retinal progenitors are observed (fig. S7). However, the absence of an effect on horizontal cells indicates that GDF11 signaling does not regulate production of all cell types in the retina. Instead, it must govern either a specific subprogram of retinal neurogenesis or act on only a subset of multipotent progenitor cells. This last idea suggests that early retinal progenitors, despite possessing the potential to give rise to all retinal cell types, are nonetheless heterogeneous, at least with respect to their capacity to respond to GDF11.

Our finding that GDF11 governs retinal progenitor cell fate without altering proliferation supports the idea—suggested by retroviral lineage studies—that regulation of cell division and cell-type determination occur independently in the retina (2). Moreover, our results highlight the difference in feedback mechanisms employed in different regions of the developing nervous system to effect proper neuron number. In retina, feedback regulation of neural cell number, mediated by GDF11 expressed by the earliest born neurons, is accomplished by altering the fates of multipotent progenitor cells independent of proliferation. In other regions, such as OE, neuronal GDF11 feeds back to regulate progenitor cell proliferation, independent of changes in cell fate (14). Finally, these studies demonstrate the diversity of action of GDF11 itself. In OE, GDF11 exerts its anti-neurogenic action by inducing reversible cell-cycle arrest in committed progenitors through increased expression of the cyclin-dependent kinase inhibitor p27Kip1 (14). In retina, by contrast, GDF11 controls the time course of expression of genes that regulate competence to produce RGCs, but neither p27Kip1 levels nor cell proliferation are affected (figs. S3 and S8). Thus, GDF11 acts as a negative feedback regulator of neurogenesis during development by altering either progenitor cell proliferation or progenitor cell fate in different tissues.

References and Notes
1. R. Wetts, S. E. Fraser, Science 239, 1142 (1988).
2. D. L. Turner, E. Y. Snyder, C. L. Cepko, Neuron 4, 833 (1990).
3. R. W. Young, Anat. Rec. 212, 199 (1985).
4. D. H. Rapaport, L. L. Wong, E. D. Wood, D. Yasumura, M. M. LaVail, J. Comp. Neurol. 474, 304 (2004).
5. T. Watanabe, M. C. Raff, Neuron 4, 461 (1990).
6. M. J. Belliveau, C. L. Cepko, Development 126, 555 (1999).
7. D. H. Rapaport, S. L. Pathel, W. A. Harris, J. Neurobiol. 49, 129 (2001).
8. M. L. Vetter, H. L. Brown, Semin. Cell Dev. Biol. 12, 491 (2001).
9. T. Marquardt, P. Gruss, Trends Neurosci. 25, 32 (2002).
10. M. Gonzalez-Hoyuela, J. A. Barbas, A. Rodriguez-Tebear, Development 128, 117 (2001).
11. T. A. Reh, T. Tully, Dev. Biol. 114, 463 (1986).
12. D. K. Waid, S. C. McLoon, Development 125, 1059 (1998).
13. M. Nakashima, T. Toyono, A. Akamine, A. Jayner, Mech. Dev. 80, 185 (1999).
14. H. H. Wu et al., Neuron 37, 197 (2003).
15. L. W. Garner et al., Dev. Biol. 208, 222 (1999).
16. S. P. Oh et al., Genes Dev. 16, 2749 (2002).
17. S. M. Federman et al., J. Bone Miner. Res. 15, 5103 (2000).
18. S. J. Lee, A. C. McPherson, Proc. Natl. Acad. Sci. U.S.A. 98, 9306 (2001).
19. M. Xiang et al., Neuron 11, 689 (1993).
20. L. Gan et al., Proc. Natl. Acad. Sci. U.S.A. 93, 3920 (1996).
21. D. K. Waid, S. C. McLoon, Neuron 14, 117 (1995).
22. S. Chen et al., Neuron 19, 1017 (1997).
23. C. J. Barnstable, R. Hofstein, K. Akagawa, Brain Res. 352, 206 (1985).
24. T. Marquardt et al., Cell 105, 43 (2001).
25. M. A. Dyer, F. J. Livesey, C. L. Cepko, G. Oliver, Nat. Genet. 34, 53 (2003).
26. J. R. Sparrow, D. Hicks, C. J. Barnstable, Brain Res. Dev. Brain Res. 51, 69 (1990).
27. S. Kanekar et al., Neuron 19, 981 (1997).
28. N. L. Brown, S. Patel, J. Brzesinski, T. Glaser, Development 128, 2497 (2001).
29. Z. Yang, K. Ding, L. Pan, M. Deng, L. Gan. Dev. Biol. 264, 240 (2003).
30. J. Hatakeyama, K. Tomita, T. Inoue, R. Kageyama, Development 128, 1313 (2001).
31. E. M. Morrow, T. Furukawa, J. E. Lee, C. L. Cepko, Development 126, 23 (1999).
32. W. Liu, J. H. Wang, M. Xiang, Dev. Dyn. 217, 320 (2000).
33. Materials and methods are available as supporting material on Science Online.
34. This work was supported by the March of Dimes Birth Defects Foundation, NIH grants DC03583 and HD38761 to A.L.C. A.D.L. is supported by HD38761, K.M.L. by AR44528, and M.M.M. by HD32067.

Supporting Online Material
www.sciencemag.org/cgi/content/full/308/5730/1927/DC1
Materials and Methods
Figs. S1 to S8

References
25 January 2005; accepted 29 April 2005
10.1126/science.1110175