Norrin mediates opposing effects on tumor progression of glioblastoma stem cells

Stefan Kassumeh, Siegfried G. Priglinger, and Andreas Ohlmann

Department of Ophthalmology, University Hospital, LMU Munich, Munich, Germany.

Glioblastoma is the most common human brain cancer entity and is maintained by a glioblastoma stem cell (GSC) subpopulation. In this issue of the *JCI*, El-Sehemy and colleagues explored the effects that Norrin, a well-characterized activator of Wnt/β-catenin signaling, had on tumor growth. Norrin inhibited cell growth via β-catenin signaling in GSCs that had low expression levels of the transcription factor ASCL1. However, Norrin had the opposite effect in GSCs with high ASCL1 expression levels. The modulation of Norrin expression, with respect to high or low ASCL1 levels in GSCs, significantly reduced tumor growth in vivo, and subsequently increased the survival rate of mice. Notably, Norrin mediates enhanced tumor growth of glioblastomas by activating the Notch pathway. This study clarifies the opposing effects of Norrin on glioblastoma tumor growth and provides potential therapeutic targets for glioblastoma treatment.

Norrin: a multifunctional signaling molecule

Norrin is a secreted signaling molecule that activates canonical Wnt/β-catenin signaling via binding to frizzled 4 (FZD4) or leucine-rich repeat–containing G protein–coupled receptor 4 (LGR4) (1–3). So far, the effects of Norrin on the vascular system and neurons in the central nervous system (CNS) as well as the retina have been well described. The angiogenic impact of Norrin on the development of retinal vasculature was first observed in Norrin-deficient mice, which showed an abnormal retinal vascularization with a disturbed inner blood-retina barrier (BRB) (4). After Xu and colleagues discovered the Norrin receptor FZD4 (3), studies revealed the roles of Norrin/β-catenin signaling in the development and maintenance of the CNS and retinal vasculature (5–7). In addition, Norrin is involved in BRB and blood-brain barrier formation as well as pathological retinal neovascularization (6–9). A complex signaling network has been established for the angiogenic role of Norrin that involves the proangiogenic transcription factor Sox17, angiogenic factors for vessel formation as well as maturation, and the coreceptor tetraspanin 12, which specifically enhances Norrin signaling (9–12).

Again, the impact of Norrin on retinal neurons was first observed in Norrin-deficient mice, which have a continuous loss of retinal ganglion cells (RGCs) (4). Vice versa, overexpression of Norrin in the eye leads to an increase in retinal neurons (13). During development, Norrin mediates signaling downstream of hedgehog, promoting retinal progenitor cells to reenter the cell cycle (14). Furthermore, the expression of Norrin in astrocytes appears to regulate the formation of neuronal dendrites and spines (15). In addition, after retinal damage Norrin mediates protective effects on RGCs and photoreceptors (16–20). The neuroprotective effects of Norrin on acutely damaged RGCs are mediated by an enhanced expression of leukemia inhibitory factor via β-catenin signaling in Müller cells (16, 20).

▶ **Related Article: p. 3069**

Opposing effects of Norrin on glioblastoma stem cells

In glioma cell lines and primary human gliomas, enhanced Norrin expression was observed when compared with other tumors. Interestingly, no difference in *FZD4* expression was detected. The enhanced expression of Norrin in gliomas encouraged El-Sehemy and colleagues (21) to correlate Norrin expression with the survival rate of patients with glioblastoma, neuroblastoma, and astrocytoma. Their results strongly suggested that Norrin has an inhibitory effect on tumor progression.

To analyze the potential underlying mechanism for the Norrin-mediated tumor-inhibitory effects, the researchers knocked down Norrin or *FZD4* expression, and overexpressed Norrin in human fetal neuronal stem cells (NSCs). Norrin and *FZD4* knockdown led to an inhibited proliferation, whereas overexpressing Norrin had the opposite effect. However, in primary patient–derived glioblastoma stem cells (GSCs), a subpopulation of glioblastoma cells, the Norrin effects on GSC proliferation and sphere formation depended on the expression of ASCL1, a basic helix-loop-helix transcription factor. In GSCs with low ASCL1 expression, the knockdown of Norrin and *FZD4* enhanced proliferation and sphere formation, whereas the overexpression of Norrin inhibited both proliferation and sphere formation via activating canonical Wnt/β-catenin signaling. However, in GSCs with high ASCL1 expression, Norrin knockdown inhibited growth, while *FZD4* knockdown failed to reduce proliferation, indicating that Norrin signaling acts independently of FZD4 in these cells. Further, in GSCs with high ASCL1 expression Norrin enhanced Notch signaling. Thus, the expression level of ASCL1 in GSCs is crucial for the opposing effects of Norrin as well as the activation of

Conflict of interest: SGP receives personal funding and travel reimbursement from Carl Zeiss Meditec AG.

Reference information: *J Clin Invest*. 2020;130(6):2814–2815. https://doi.org/10.1172/JCI137254.

different downstream pathways. Intriguingly, in vivo experiments in mice revealed that modulating Norrin transcription in GSCs with high or low ASCL1 expression affects tumor progression and leads to increased survival rate (21).

Even though the molecular interactions between Norrin and Notch signaling remain unclear, El-Sehemy and colleagues clearly demonstrate that Norrin-mediated Notch signaling, independent of FZD4, promotes tumor progression in GSCs, whereas Norrin-activated canonical Wnt/β-catenin signaling leads to an opposite effect (21).

Unanswered questions and future directions

The findings of El-Sehemy and colleagues fundamentally contribute to the understanding of glioblastoma tumor biology and of the Norrin-mediated signaling network (21). The authors used state-of-the-art technologies to analyze the molecular mechanism of Norrin-induced cell proliferation in glioblastoma and the underlying β-catenin–independent Notch signaling mechanism. Moreover, El-Sehemy and colleagues demonstrated that, depending on the expression level of ASCL1, reduced as well as enhanced Norrin expression can lead to an increased survival rate of mice (21). Although there are significant technical challenges in the treatment of patients using siRNA, modulating Norrin signaling could provide a promising option to treat glioblastoma. It would be helpful to analyze the underlying mechanism of Norrin-mediated Notch signaling in detail to find further options to block this signaling cascade.

As mentioned previously, Norrin promotes neural progenitor self-renewal and increases the number of retinal neurons in transgenic mice (13, 14). Because Notch signaling is involved in the development and regeneration of the retina and several other tissues (22, 23), one question that should be addressed is whether Norrin mediates these functions in retinal progenitors via the activation of Notch signaling and whether this potential downstream signaling of Norrin has protective or regenerative effects on retinal neurons.

Intriguingly, in the retina, Notch signaling inhibits vascular development and the formation of pathological neovascularization (for a review see ref. 24). Because Norrin has opposing effects on retinal vasculature, it is tempting to speculate whether Norrin could enhance Notch signaling in retinal microvascular endothelial cells as a negative feedback loop to avoid excessive vessel formation.

Overall, the findings of El-Sehemy and colleagues (21) are a milestone for a better understanding of glioblastoma tumor biology and Norrin signaling.

Acknowledgments

We would like to thank the Deutsche Forschungsgemeinschaft (Research Unit FOR 1075, TP 7; KA 5075/2-1; OH 214/4-3) and the Edith-Haberland-Wagner Foundation for funding our research.

Address correspondence to: Andreas Ohlmann, Department of Ophthalmology, University Hospital, LMU Munich, Mathildenstrasse 8, 80336 Munich, Germany. Phone: 49.89.440053054; Email: andreas.ohlmann@med.uni-muenchen.de.

1. Deng C, Reddy P, Cheng Y, Luo CW, Hsiao CL, Hsueh AJ. Multi-functional norrin is a ligand for the LGR4 receptor. *J Cell Sci*. 2013;126(pt 9):2060–2068.
2. Lai MB, et al. TSPAN12 is a Norrin co-receptor that amplifies Frizzled4 ligand selectivity and signaling. *Cell Rep*. 2017;19(13):2809–2822.
3. Xu Q, et al. Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell*. 2004;116(6):883–895.
4. Richter M, Gottanka J, May CA, Welge-Lüssen U, Berger W, Lütjen-Drecoll E. Retinal vasculature changes in Norrie disease mice. *Invest Ophthalmol Vis Sci*. 1998;39(12):2450–2457.
5. Zhang C, Lai MB, Khandan L, Lee LA, Chen Z, Junge HJ. Norrin-induced Frizzled4 endocytosis and endo-lysosomal trafficking control retinal angiogenesis and barrier function. *Nat Commun*. 2017;8:16050.
6. Zhou Y, et al. Canonical WNT signaling components in vascular development and barrier formation. *J Clin Invest*. 2014;124(9):3825–3846.
7. Wang Y, Rattner A, Zhou Y, Williams J, Smallwood PM, Nathans J. Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity. *Cell*. 2012;151(6):1332–1344.
8. Tokunaga CC, Chen YH, Dailey W, Cheng M, Drenser KA. Retinal vascular rescue of oxygen-induced retinopathy in mice by norrin. *Invest Ophthalmol Vis Sci*. 2013;54(1):222–229.
9. Ohlmann A, Seitz R, Braunger B, Seitz D, Bösl MR, Tamm ER. Norrin promotes vascular regrowth after oxygen-induced retinal vessel loss and suppresses retinopathy in mice. *J Neurosci*. 2010;30(1):183–193.
10. Ye X, et al. Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization. *Cell*. 2009;139(2):285–298.
11. Junge HJ, et al. TSPAN12 regulates retinal vascular development by promoting Norrin- but not Wnt-induced FZD4/beta-catenin signaling. *Cell*. 2009;139(2):299–311.
12. Zeilbeck LF, et al. Norrin mediates angiogenic properties via the induction of insulin-like growth factor-1. *Exp Eye Res*. 2016;145:317–326.
13. Ohlmann A, et al. Ectopic norrin induces growth of ocular capillaries and restores normal retinal angiogenesis in Norrie disease mutant mice. *J Neurosci*. 2005;25(7):1701–1710.
14. McNeill B, et al. Hedgehog regulates Norrie disease protein to drive neural progenitor self-renewal. *Hum Mol Genet*. 2013;22(5):1005–1016.
15. Miller SJ, et al. Molecularly defined cortical astroglia subpopulation modulates neurons via secretion of Norrin. *Nat Neurosci*. 2019;22(5):741–752.
16. Seitz R, Hackl S, Seibuchner T, Tamm ER, Ohlmann A. Norrin mediates neuroprotective effects on retinal ganglion cells via activation of the Wnt/beta-catenin signaling pathway and the induction of neuroprotective growth factors in Muller cells. *J Neurosci*. 2010;30(17):5998–6010.
17. Leopold SA, et al. Norrin protects optic nerve axons from degeneration in a mouse model of glaucoma. *Sci Rep*. 2017;7(1):14274.
18. Lin S, Cheng M, Dailey W, Drenser K, Chintala S. Norrin attenuates protease-mediated death of transformed retinal ganglion cells. *Mol Vis*. 2009;15:26–37.
19. Kassumeh S, et al. Norrin protects retinal ganglion cells from excitotoxic damage via the induction of leukemia inhibitory factor. *Cells*. 2020;9(2):E277.
20. El-Sehemy A, et al. Norrin mediates tumor-promoting and -suppressive effects in glioblastoma via Notch and WNT. *J Clin Invest*. 2020;130(6):3069–3086.
21. Mills EA, Goldman D. The regulation of Notch signaling in retinal development and regeneration. *Curr Pathobiol Rep*. 2017;5(4):323–331.
22. Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. *Physiol Rev*. 2017;97(4):1235–1294.
23. Lobov I, Mikhailova N. The role of Dll4/Notch signaling in normal and pathological ocular angiogenesis: Dll4 controls blood vessel sprouting and vessel remodeling in normal and pathological conditions. *J Ophthalmol*. 2018;2018:3565292.