Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Lipid Raft-Excluded GFR α 1/Ret Fails to Transmit GDNF Signaling *In Vivo*

Daniel Morales

Institut de recherches cliniques de Montréal, Montréal, Québec, H2W 1R7, Canada and Integrated Program in Neuroscience, McGill University, Montréal, Québec, H3A 2B4, Canada

Review of Tsui et al.

Many years of observations, beginning with the study of asymmetric cellular lipid trafficking and driven primarily by the technique of cold detergent extraction and membrane fractionation, led to the formulation of the lipid raft hypothesis (Simons and Van Meer, 1988; Lingwood and Simons, 2010). This posits that there exist, floating on the plasma membrane, islands rich in sphingolipids and cholesterol that function as tiny signaling hubs, able to concentrate specific receptors and downstream effectors and possibly even exclude unwanted molecules (Simons and Ikonen, 1997; Pike, 2006). These capacities are thought to confer greater control over the spatial and temporal activation of signaling, for example, by selectively recruiting key components of signaling machinery to the raft (Pike, 2003). Acceptance of this idea was not immediate, however, because rafts have been difficult to directly observe and most evidence for their existence has

come from *in vitro* experiments (Munro, 2003).

Nonetheless, many persuasive arguments for lipid rafts as functional membrane compartments exist, some of them emerging from the study of glial cell line-derived neurotrophic factor (GDNF). GDNF is an important neuronal survival factor (Moore et al., 1996), and an axon guidance cue for limb-innervating spinal motor neurons (Kramer et al., 2006; Dudanova et al., 2010; Bonanomi et al., 2012). A diffusible ligand, GDNF binds to a glycosylphosphatidylinositol (GPI)-anchored receptor, GDNF family receptor α 1 (GFR α 1), that is found within membrane fractions that are thought to contain lipid rafts. GFR α 1 requires a transmembrane coreceptor, the receptor tyrosine kinase Ret, to transmit its signal intracellularly, and GDNF:GFR α 1 binding is believed to recruit Ret into rafts (Tansey et al., 2000; Paratcha et al., 2001). Indeed, the phenotypes of mice lacking GDNF are almost identical to those of mice lacking either GFR α 1 or Ret (Schuchardt et al., 1994; Moore et al., 1996; Enomoto et al., 1998). The importance of lipid rafts in this signaling pathway has been explored with the creation of a chimeric GFR α 1 in which the GPI-anchor was replaced with a transmembrane domain that excluded the receptor from the raft-containing membrane fraction (Tansey et al., 2000). Strikingly, this modified GFR α 1 was unable to properly transmit GDNF signaling despite intact ligand-binding and Ret-binding capabilities (Tansey et al.,

2000). This supports the hypothesis that the lipid raft microenvironment is required for the pathway to function properly. However, *in vivo* evidence for this model of GDNF signaling was missing.

In a recent issue of *The Journal of Neuroscience*, Tsui and colleagues (2015) addressed this gap by creating a knock-in mouse in which the lipid raft-excluded transmembrane GFR α 1 replaced its wild-type version (Tansey et al., 2000; Tsui et al., 2015). The resulting mouse, termed GFR α 1-TM, had normal levels of GFR α 1 expression, and its trafficking to the plasma membrane was unchanged. The authors also confirmed, in whole embryo homogenates and primary sensory neuron cultures, that GFR α 1-TM was able to bind Ret and induce its autophosphorylation, just like wild-type GFR α 1. However, when sensory neurons from GFR α 1-TM mice were stimulated with GDNF, Ret did not relocate to lipid raft membrane fractions. These observations argue that the GDNF:GFR α 1-TM/Ret complex forms normally on the plasma membrane, and that it is only its distribution within membrane subdomains that is changed.

Just how important is it to localize this signaling complex within lipid rafts? Strikingly, the GFR α 1-TM mouse phenocopies the GFR α 1 knock-out. Homozygous knock-in mice failed to develop kidneys and enteric neurons, a phenotype associated with genetic loss of the GDNF pathway (Schuchardt et al., 1994; Moore et al., 1996; Enomoto et al., 1998). The

Received Nov. 18, 2015; revised Dec. 18, 2015; accepted Dec. 29, 2015.

D.M. is supported by an international graduate fellowship from the Mexican Council of Science and Technology (CONACYT). The author wishes to thank Dr. Artur Kania for his supervision and for providing critical comments on an earlier version of this article. Dr. Dario Bonanomi and Dr. Stefania Averaimo shared many helpful insights while this manuscript was being prepared, which were much appreciated.

Correspondence should be addressed to Daniel Morales, Institut de recherches cliniques de Montréal, 110 Avenue des Pins Ouest, Montréal, QC, H2W 1R7, Canada. E-mail: daniel.morales@ircm.qc.ca.

DOI:10.1523/JNEUROSCI.4155-15.2016

Copyright © 2016 the authors 0270-6474/16/361442-03\$15.00/0

total penetrance of the phenotype—all homozygous *GFR α 1-TM* mice observed lacked kidneys—is a dramatic demonstration that the lipid raft-excluded receptor is useless in these tissues.

How is it possible to block signaling despite having spared ligand/receptor complex formation on the membrane? One answer was suggested by studies in cultured sympathetic neurons, where the survival-promoting effects of GDNF were abolished by pharmacologically disrupting lipid rafts, but were restored by inhibiting the proteasome (Pierchala et al., 2006). Lipid rafts were thus suggested to sequester Ret away from degradation, prolonging its signaling half-life. Tsui and colleagues (2015) investigated Ret ubiquitination, a first step in proteasome-mediated degradation, and found that levels were significantly higher in whole-brain extracts of *GFR α 1-TM* mice than in controls, providing *in vivo* evidence for this model.

A different mechanism was suggested by an earlier study that showed that binding between Ret and lipid raft-associated Src family kinases (SFKs) is vastly diminished in cells expressing *GFR α 1-TM* compared with those expressing wild-type receptor (Tansey et al., 2000). This Ret-SFK interaction was shown to be required for GDNF signaling (Encinas et al., 2001), arguing that the presence of the receptor complex in lipid rafts brings Ret into contact with critical effector molecules that cannot be accessed otherwise. Levels of association between Ret and SFKs, or other downstream effectors, were not determined in the knock-in mouse.

These lipid raft-dependent signaling mechanisms have important implications for the field of axon guidance. Spinal motor axon innervation of the dorsal limb mesenchyme is controlled in part by GDNF:GFR α 1/Ret signaling (Kramer et al., 2006; Dudanova et al., 2010). In addition to expressing GDNF, the dorsal limb tissue is rich in EphA, and motor axons innervating it express its binding partner, GPI-anchored ephrinA, which is also thought to reside in lipid rafts (Bonanomi et al., 2012). EphA:ephrinA signaling also depends on Ret as a coreceptor, and the coincidence of GDNF and EphA produce synergistic attractive growth cone responses (Bonanomi et al., 2012). In *GFR α 1-TM* mouse limbs, the dorsal nerve fails to grow as axons are all redirected ventrally, a phenotype that has previously been reported in mice with defective GDNF or EphA pathways (Helmbacher et

al., 2000; Kramer et al., 2006; Gould et al., 2008; Bonanomi et al., 2012).

Although the molecular details of GDNF-Eph cooperation in growth cones remain unclear, the phenotype observed in the *GFR α 1-TM* mouse is consistent with what had been suggested by the membrane fractionation experiments of Bonanomi and colleagues (2012): the signal integration mechanism of GDNF and Eph ligands likely occurs in membrane fractions that contain lipid rafts (Bonanomi et al., 2012). Could the synergistic effects be explained by raft-dependent mechanisms? For example, could the lipid raft environment play a role in preventing Ret degradation by excluding signal-termination machinery? A more tentative hypothesis is that lipid rafts containing GDNF and/or EphA pathway components fuse together upon coincidence of both ligands, thus bringing in close proximity downstream effectors that are kept apart under basal conditions, or when only one stimulus is present. This could potentiate the canonical signaling pathway of each ligand through enhanced effector availability or through feedforward loops, or could activate a signaling cascade exclusive to ligand coincidence. Either way, the characteristics associated with lipid rafts make them the perfect structures for modulating the signaling thresholds of the pathways, and therefore for mediating growth cone attraction to concentrations of ligand that elicit no response when presented alone. Raft-mediated mechanisms like those proposed here could well be playing a role in other axonal choice points, since multiple overlapping axon guidance cues seem to be the rule rather than the exception (Dudanova and Klein, 2013).

There's a more general question raised by the results of Tsui and colleagues (2015). Lipidated proteins are thought to cluster in rafts due to their biophysical properties, but the mechanism that recruits outside proteins remains poorly understood. Ret, for example, is mostly found outside lipid rafts (Tansey et al., 2000; Paratcha et al., 2001), but this may be more because rafts represent a small component of the membrane than because it is actively excluded. Recruited proteins may be allowed to freely move in and out of rafts under basal conditions, but upon specific signaling events, they may be "caught" and maintained by binding to lipid raft-associated proteins whose conformation has changed, like GDNF-bound GFR α 1 or myristoylated FRS2 in

the case of Ret (Tansey et al., 2000; Paratcha et al., 2001).

Despite the gaps that remain in our knowledge, the study by Tsui and colleagues (2015) is a compelling demonstration of a receptor complex whose signaling depends on its translocation to lipid rafts. As such, it will stand as an important case for their functional relevance. Future work will hopefully address some of the central questions this study raises: Is the effect of lipid raft targeting only to protect molecules from degradation and to enable effector binding, or is there more? Are rafts in general playing a major role in mediating the integration of overlapping signaling systems? In the case of GDNF, the requirement for rafts seems to be absolute, but for other molecular pathways, lipid rafts may serve more of a modulatory function, amplifying or dampening their signals. Could this be a way to make cellular responses robust in the face of noisy expression levels of signaling components? The *GFR α 1-TM* mouse demonstrates the power of subtle modifications of molecules for answering biological questions like these. Similar tinkering with receptors or downstream effectors can be easily done using CRISPR-mediated genome editing, for example. This approach is sure to have much more to teach us, not only about the role of lipid rafts, but about cellular signaling in general.

References

- Bonanomi D, Chivatakarn O, Bai G, Abdesslem H, Lettieri K, Marquardt T, Pierchala BA, Pfaff SL (2012) Ret is a multifunctional coreceptor that integrates diffusible- and contact-axon guidance signals. *Cell* 148:568–582. [CrossRef Medline](#)
- Dudanova I, Gatto G, Klein R (2010) GDNF acts as a chemoattractant to support ephrinA-induced repulsion of limb motor axons. *Curr Biol* 20:2150–2156. [CrossRef Medline](#)
- Dudanova I, Klein R (2013) Integration of guidance cues: parallel signaling and crosstalk. *Trends Neurosci* 36:295–304. [CrossRef Medline](#)
- Encinas M, Tansey MG, Tsui-Pierchala BA, Comella JX, Milbrandt J, Johnson EM Jr (2001) c-Src is required for glial cell line-derived neurotrophic factor (GDNF) family ligand-mediated neuronal survival via a phosphatidylinositol-3 kinase (PI-3K)-dependent pathway. *J Neurosci* 21:1464–1472. [Medline](#)
- Enomoto H, Araki T, Jackman A, Heuckeroth RO, Snider WD, Johnson EM Jr, Milbrandt J (1998) GFR α 1-deficient mice have deficits in the enteric nervous system and kidneys. *Neuron* 21:317–324. [CrossRef Medline](#)
- Gould TW, Yonemura S, Oppenheim RW, Ohmori S, Enomoto H (2008) The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are

- restricted to fusimotor subtypes. *J Neurosci* 28:2131–2146. [CrossRef Medline](#)
- Helmbacher F, Schneider-Maunoury S, Topilko P, Tiet L, Charnay P (2000) Targeting of the EphA4 tyrosine kinase receptor affects dorsal/ventral pathfinding of limb motor axons. *Development* 127:3313–3324. [Medline](#)
- Kramer ER, Knott L, Su F, Dessaud E, Krull CE, Helmbacher F, Klein R (2006) Cooperation between GDNF/Ret and ephrinA/EphA4 signals for motor-axon pathway selection in the limb. *Neuron* 50:35–47. [CrossRef Medline](#)
- Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. *Science* 327:46–50. [CrossRef Medline](#)
- Moore MW, Klein RD, Fariñas I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carver-Moore K, Rosenthal A (1996) Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 382:76–79. [CrossRef Medline](#)
- Munro S (2003) Lipid rafts: elusive or illusive? *Cell* 115:377–388. [CrossRef Medline](#)
- Paratcha G, Ledda F, Baars L, Couplier M, Besset V, Anders J, Scott R, Ibáñez CF (2001) Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts. *Neuron* 29:171–184. [CrossRef Medline](#)
- Pierchala BA, Milbrandt J, Johnson EM Jr (2006) Glial cell line-derived neurotrophic factor-dependent recruitment of Ret into lipid rafts enhances signaling by partitioning Ret from proteasome-dependent degradation. *J Neurosci* 26:2777–2787. [CrossRef Medline](#)
- Pike LJ (2003) Lipid rafts: bringing order to chaos. *J Lipid Res* 44:655–667. [CrossRef Medline](#)
- Pike LJ (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J Lipid Res* 47:1597–1598. [CrossRef Medline](#)
- Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F, Pachnis V (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature* 367:380–383. [CrossRef Medline](#)
- Simons K, van Meer G (1988) Lipid sorting in epithelial cells. *Biochemistry* 27:6197–6202. [CrossRef Medline](#)
- Simons K, Ikonen E (1997) Functional rafts in cell membranes. *Nature* 387:569–572. [CrossRef Medline](#)
- Tansey MG, Baloh RH, Milbrandt J, Johnson EM Jr (2000) GFRalpha-mediated localization of RET to lipid rafts is required for effective downstream signaling, differentiation, and neuronal survival. *Neuron* 25:611–623. [CrossRef Medline](#)
- Tsui CC, Gabreski NA, Hein SJ, Pierchala BA (2015) Lipid rafts are physiologic membrane microdomains necessary for the morphogenic and developmental functions of glial cell line-derived neurotrophic factor in vivo. *J Neurosci* 35:13233–13243. [CrossRef Medline](#)