

## Psychosine, Cytokinesis, and Orphan Receptors: Unexpected Connections

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One of the most exciting aspects of modern cell biology is the potential to make connections between disparate areas of research, and thus open up new lines of enquiry. Two recent papers have done just this (Kanazawa et al., 2000; Im et al., 2001, this issue). Building on the biochemical defect in an inherited metabolic disease (Suzuki, 1998), these papers reveal an unexpected connection between lipid mediators, G protein-coupled receptor (GPCR)<sup>1</sup> signaling, and cytokinesis mechanism.

Globoid cell leukodystrophy (GLD), or Krabbe's disease, is a severe, inherited, metabolic disorder in which normal myelin formation is blocked, and multinucleate "globoid cells" accumulate in the brain. The primary defect is an absence of the lipid-degrading enzyme galactocereamidase, which cleaves the galactose headgroup from galactoceramide (Fig. 1). Galactoceramide-deficient mice and dogs provide models for human GLD (Suzuki, 1998). Unlike some other lipidoses, the primary substrate of the missing enzyme, galactoceramide, does not accumulate. Instead, a related lipid metabolite, psychosine (Fig. 1), accumulates in the brain (Svennerholm et al., 1980). Suzuki (1998) hypothesized that psychosine is normally broken down by galactoceramidase, and that in its absence psychosine accumulates, causing death of oligodendrocytes. These are the cells that normally synthesize galactoceramide during myelination, so their death would account for the absence of galactoceramide buildup, and also GLD pathology. This "psychosine hypothesis" has stood the test of time, though the mechanism by which psychosine might mediate toxic effects was unknown. The multinucleate globoid cells in GLD are thought to derive from microglia and macrophages (Kanazawa et al., 2000), but the reason they accumulate was unknown.

To test the hypothesis that accumulated psychosine might trigger formation of multinucleate globoid cells, Kanazawa et al. (2000) treated several cell lines with psychosine at concentrations relevant to GLD. They found that a premonocyte-like cell line, U937, responded by becoming multinucleate over a few days. Cytochemistry and time-lapse imaging of dividing cells showed that psychosine did not induce cell fusion; rather, it blocked cytokine-

sis, uncoupling mitosis from cell division. This discovery is the first reported inhibition of cytokinesis by a physiologically occurring small molecule, and a large step forward in understanding the pathogenesis of GLD. However, it was not clear how the target of psychosine might be discovered. Psychosine is a lysolipid, with detergent-like properties, so its target might in principle be some aspect of membrane biophysics rather than a specific protein.

While hunting for the ligands of an orphan receptor, Im et al. (2001) appear to have identified one target of psychosine. It is a GPCR called TDAG8, previously named for its high expression in T cells undergoing apoptosis (Choi et al., 1996), but otherwise uncharacterized. Discovering the ligands for "orphan" GPCRs and nuclear receptors (receptors identified by sequence for which ligands and function are unknown) is an important endeavor. GPCRs are involved in regulating many aspects of physiology, and GPCR agonists and antagonists constitute one of the largest categories of therapeutic drugs. Orphan GPCRs may thus be the targets for drugs of the future. To understand the physiological role of a GPCR, and obtain the chemical starting point for drug design, it is important to identify its physiological ligand (Wilson et al., 1998; Sautel and Milligan, 2000). GPCRs are notable for the diversity of their ligands, including proteins, small molecules, and even photons. An important class of GPCR ligands is lipid mediators, metabolites of common lipids that play important and diverse roles in signaling between and within cells. Considerable effort has been devoted to identifying the presumed GPCR receptors of sphingosine containing lipid mediators. Recently, a GPCR receptor for sphingosylphosphorylcholine (Fig. 1), named OGR1, was identified (Xu et al., 2000). Im et al. (2000, 2001) were also interested in identifying new sphingosine receptors, and they expressed in cells the orphan GPCR, TDAG8, that is 41% identical to OGR1, with the expectation that its ligand might be a lipid related to sphingosylphosphorylcholine. This lipid is structurally related to psychosine; both are lyso-sphingolipids; that is, sphingolipids that lack the second fatty acid normally attached as an amide to the amino group of sphingosine (Fig. 1).

Using standard tests for GPCR activation, changes in intracellular [cAMP] and [Ca<sup>2+</sup>], Im et al. (2001) found that TDAG8 is activated by psychosine and related lysosphingolipids. Primed by the Kanazawa et al. (2000) paper, they went on to show that expression of TDAG8 in a cell that

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<sup>1</sup>Abbreviations used in this paper: GLD, globoid cell leukodystrophy; GPCR, G protein-coupled receptor.

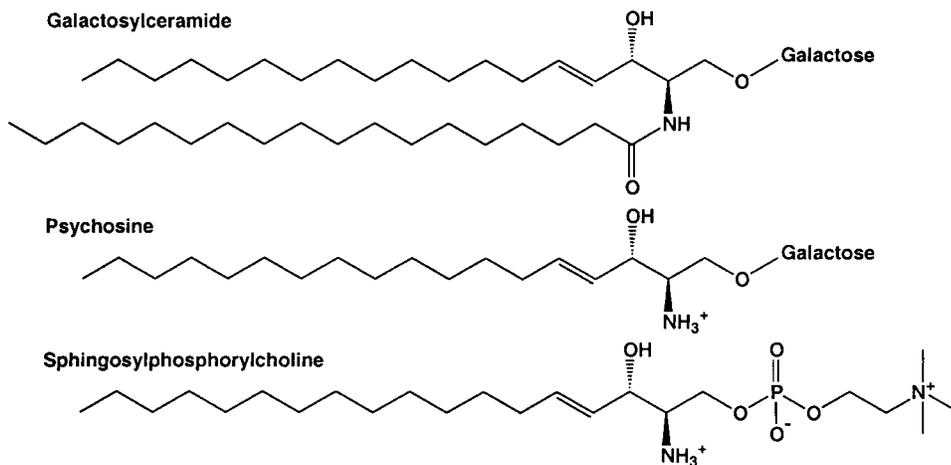


Figure 1. Structures of lipids.

does not normally express it, together with the addition of psychosine, promotes accumulation of multinucleate cells. Completing the connection, Im et al. (2001) showed that the U937 cells shown by Kanazawa et al. (2000) to become multinucleate in response to psychosine express TDAG8 endogenously, whereas other cell lines that do not respond do not express this receptor. Im et al. (2001) concluded that TDAG8 is the receptor for psychosine and a cytokinesis blocker.

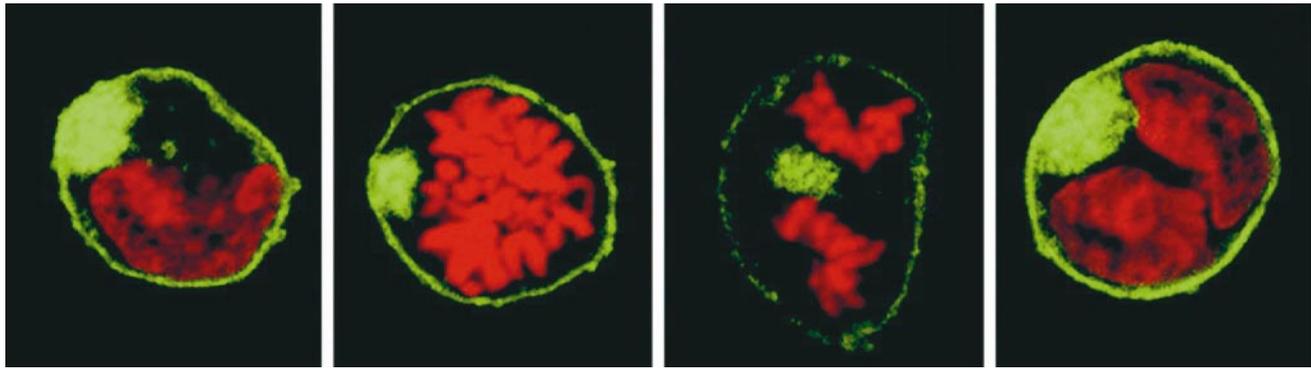
Before exploring the implications, it is worth critiquing this interesting conclusion. The  $K_i$  for psychosine in decreasing [cAMP] and increasing [Ca<sup>2+</sup>] in TDAG8-expressing cells was  $\sim 3 \mu\text{M}$ , significantly higher than the  $K_i$  for some other signaling lipids binding to their GPCR receptors. For example, sphingosylphosphorylcholine activates the related GPCR OGR1 with a  $K_i$  of 30 nM. The relatively low apparent affinity of psychosine for TDAG8 is consistent with TDAG8 being the psychosine receptor in GLD, where the psychosine accumulates to high concentrations. But it argues caution in concluding that psychosine is the normal physiological ligand for TDAG8. A second potential concern is that psychosine might have additional effects that are required in addition to activating TDAG8 for blocking cytokinesis. Lysolipids are cone shaped, compared with the more cylindrical shape of lipids with two hydrocarbon chains, and  $\mu\text{M}$  concentrations of psychosine might alter the biophysics of the plasma membrane in addition to activating TDAG8. Suzuki (1998) notes that psychosine causes death of many cell types in culture with a threshold-like dose-response curve, potentially consistent with detergent-like effects.

Assuming that psychosine binding to TDAG8 alone is sufficient to block cytokinesis, what are the implications? Several scenarios, not mutually exclusive, can be considered. This ligand-receptor pair may represent a physiological pathway for regulating cytokinesis, as suggested by Im et al. (2001). Several cell types in mammals become multinucleate as part of their normal developmental program, including trophoblast cells, megakaryocytes, and osteoclasts. In the case of megakaryocytes, observation in culture has shown that this is due to uncoupling of mitosis from cytokinesis (Nagata et al., 1997). Psychosine and TDAG8 could be involved in activating a physiological multinucleation pathway. The tissue expression of

TDAG8 (Im et al., 2001) is consistent with such a hypothesis, though direct experiment will be needed to test it.

The discovery of a receptor for psychosine, and development of a cell-based assay for its activation, open the door to the medicinal chemistry of this class of lipid mediator. The pharmaceutical industry has an impressive track record of developing high affinity, reasonably specific agonists and antagonists for GPCRs once this information is available (Wilson et al., 1998; Sautel and Milligan, 2000). Im et al. (2001) suggest that psychosine antagonists might have therapeutic potential for treating GLD and perhaps other lipidoses. Given the massive accumulation of psychosine in GLD, it seems likely that providing an exogenous source of galactoceramide might be required in addition to a psychosine antagonist to treat the disease effectively.

What are the implications of these papers for cytokinesis mechanism? An important issue is the specificity of the psychosine/TDAG8 effect on cytokinesis. At the level of the receptor, the cytokinesis block must be specific. GPCRs are ubiquitous in mammalian physiology, but multinucleate cells are rare. An endogenous GPCR receptor for a different lysosphingolipid, sphingosylphosphorylcholine, is expressed in the RH7777 cells used by Im et al. (2001) for TDAG8 expression (Im et al., 2000). Stimulation of these cells with sphingosylphosphorylcholine caused a decrease in [cAMP] comparable to the psychosine/TDAG8 effect, but no induction of multinucleated cells. This argues that the effect of psychosine/TDAG8 on cytokinesis is not simply due to its effects on [cAMP] or [Ca<sup>2+</sup>]. More likely, the cytokinesis effect is triggered by some pertussis toxin-resistant heterotrimeric GTPase that is specifically coupled to TDAG8. Identification of this GTPase will be important for mechanistic follow up of the psychosine effect. At the level of cortical response to psychosine/TDAG8, specificity is less clear, and activated TDAG8 may effect more than one aspect of cortical dynamics. To test for an effect of psychosine on other aspects of cortical dynamics, Kanazawa et al. (2000) measured phagocytosis. The weak phagocytic activity of undifferentiated U937 cells, now known to express TDAG8, was inhibited by psychosine. The much stronger phagocytic activity of phorbol ester differentiated U937 was not affected by psychosine. However, it is not clear whether the



**Figure 2.** DNA (red) and actin (green) staining of psychosine-treated U937 cells. These cells are now known to express TDAG8 (Im et al., 2001). The images show sequential stages of failed cytokinesis in fixed cells. Note the large “clot” of actin present in all the images. At the EM level this clot contains many small vesicles. Reproduced from Kanazawa et al. (2000) with permission.

differentiated U937 cells still express TDAG8, so the implications are inconclusive. Cytological observation by Kanazawa et al. (2000) revealed one major effect of psychosine on the interphase cortex. Large “clots” of F-actin formed in undifferentiated, interphase U937 cells exposed to psychosine (Fig. 2). These clots were present in mononucleate cells, so they are not simply a consequence of failed cytokinesis. At the EM level, the actin clots were associated with clusters of vacuoles near the plasma membrane. The vacuoles were reminiscent of some endocytic compartment, and endosomes are known to associate with actin (for example, Taunton et al., 2000). These observations argue for effects of psychosine/TDAG8 on interphase cortical dynamics in addition to their effect on cytokinesis. It is even possible that the block to cytokinesis induced by psychosine/TDAG8 is a secondary effect, due to the actin clot interfering with cytokinesis. Within cytokinesis, time-lapse observation showed psychosine-treated cells initiating furrows, but failing to complete cytokinesis (Kanazawa et al., 2000). Instead the furrow moved to an off-center position and regressed. Failure to complete cytokinesis is a common phenotype of mutations in proteins required for cytokinesis, and can probably arise from several distinct problems, including defects in contractile ring assembly or force generation, defects in new membrane insertion, and defects in midbody assembly (Glotzer, 1997). As psychosine/TDAG8 causes an accumulation of actin-surrounded vacuoles in interphase U937 cells, the effect on cytokinesis might be a block to normal membrane insertion at the furrow. For example, the signaling pathways triggered by psychosine/TDAG8 might interfere with those required for coordinated contraction and membrane insertion during cytokinesis.

More work is required to address the mechanism by which psychosine/TDAG8 affects cytokinesis, and perhaps other cortical processes, and to determine if this pathway plays a role in physiological formation of multinucleate cells. The role of toxic lipid mediators in other lipidoses is also an open question (Suzuki, 1998). The connection that

has now been established between psychosine, TDAG8, and cytokinesis has already opened new lines of investigation in these previously unlinked areas. Following these is likely to shed new light on the complex intersection of lipid-based signaling pathways and cortical dynamics involving the actin cytoskeleton and membranes. This intersection is likely to provide a rich vein for both basic cell biology and pharmaceutical research to mine in the future.

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