Sphingolipids and Transient Receptor Potential Potential Channels
Evolutionarily Ancient Families Now Joined

J. Marc Simard, Volodymyr Gerzanich

Sphingolipids are one of three main classes of lipids in the cell membrane, the others being glycerolipids and sterols. Whereas sphingolipids were initially considered to act only as structural components of biological membranes, recent studies have shown that they also act as both first and second messengers in a variety of signaling pathways and have important roles in membrane microdomains called “lipid rafts.” Like the other classes of lipids, sphingolipids exhibit an enormous combinatorial structural diversity that enables functional specialization. At present, functional roles in signal transduction pathways have been elucidated mainly for the simpler sphingolipids, ceramide, sphingosine, and sphingosine-1-phosphate (S1P).

The sphingolipid metabolite, S1P, is an evolutionarily ancient signaling molecule that functions in plants, yeast, and mammals. Its conserved actions across phylogenetic systems is consistent with important biological roles. In higher eukaryotes, S1P is the ligand for a family of 5 G protein–coupled receptors. These S1P receptors are differentially expressed, coupled to various G proteins, and regulate angiogenesis, vascular maturation, cardiac development, and immunity, and are important for directed cell movement.

Similarly, the transient receptor potential (TRP) proteins, originally discovered in the fruit fly Drosophila, comprise a family of evolutionarily ancient effector molecules. TRP channels are activated by diverse non–voltage-dependent mechanisms, and appear to form many of the non–voltage-gated cationic channels found in a variety of cells. They mediate transmembrane flux of cations down their electrochemical gradients, thereby raising intracellular Na\(^+\) and Ca\(^{2+}\) concentrations, resulting in depolarization of the cell and activation of effector proteins sensitive to a rise in intracellular Ca\(^{2+}\). As many as 28 channel subunit genes are subdivided into 7 subfamilies, with each subfamily containing at least one vertebrate representative.

In vertebrates, TRP channels are implicated in numerous cellular events, including transcriptional regulation, proliferation, and directed cell movement, including neuronal growth cone steering and vascular smooth muscle cell migration.

In recent years, we have observed the rapid parallel development of understanding of the importance of sphingolipids and TRP channels in vascular biology, but until now, there has been little evidence that they might be functionally linked. Lack of evidence for a connection should perhaps not be surprising, given the tremendous complexity of each system, the large number of members in each family, and the virtual absence of specific high-affinity pharmacological agents with which to manipulate them.

In the current issue of this journal, David Beech’s laboratory demonstrates that in fact, these two families are functionally linked. They screened potential lipid regulators using HEK293 cells that stably overexpress TRPC5. In a well-designed series of experiments, they show that S1P, acting through a G protein–dependent mechanism, activates a membrane current conducted by ion channels formed by TRPC5, increasing intracellular Ca\(^{2+}\) concentration. In addition, using smooth muscle cells from saphenous veins of patients undergoing coronary artery surgery, they show that antibody-based inhibition of TRPC5 prevents S1P-induced motility of cultured smooth muscle cells. This article firmly establishes that the two families, the sphingolipids and TRP channels, are functionally linked in vascular smooth muscle.

Does this exciting finding of Beech and colleagues translate to native systems under pathological conditions? A tantalizing hint that S1P and TRPC5 may in fact interact under natural physiological conditions emerges from an analysis of the promoter regions of sphingosine kinase (SK) 1, SK2, and TRPC5. SK1 and SK2 are key enzymes responsible for phosphorylation of sphingosine to S1P, thus placing them at a critical regulatory checkpoint in S1P signaling. Analysis of the promoter regions of the mouse genes that encode SK1, SK2, and TRPC5 reveals that all three possess putative high-probability binding sites for two different transcription factor “networks,” one consisting of EGRF, HEAT, and PAX5 families, and the other consisting of ETSF and NF-\(\kappa\)B families. (A “network” is comprised of two or more transcription factor binding sites arranged in a defined order, orientation, and distance-range between adjacent sites.) Commonality of inducible transcriptional regulation for different genes suggests that, under certain conditions, the gene products will be coexpressed. Put simply, a shared transcriptional program hints that SK1, SK2, and/or TRPC5 may be expressed “at the same place, at the same time.”

Apart from putative binding sites for these two transcription factor networks, the promoter regions for the three proteins all have multiple consensus sequences for the E2F transcription factor family. The E2F family plays a central role in regulating cellular proliferation by controlling the expression of genes required for cell cycle progression, particularly DNA synthesis, and apoptosis. This family of transcription factors plays a key role in mediating hypertrophy, neointimal hyperplasia, and atherosclerosis. Indeed, E2F-decoy oligodeoxynucleotide has been under intense investigation for some time for prevention of
restenosis after coronary artery bypass graft\textsuperscript{12,13}—precisely the same procedure that yielded the human vein material used by Beech and colleagues to make their discovery.

The promoter regions for the three proteins also have multiple consensus sequences for the transcription factors, Egr-1 or 2. In an excellent, very recent review in this journal by Khachigian,\textsuperscript{14} we learn that Egr-1 is a master switch regulating cardiovascular pathology. In the vessel wall, Egr-1 is activated by mechanical injury, angiotensin II, and by several growth factors. Transcripts for Egr-1 are strikingly upregulated in both human and murine atherosclerotic lesions.\textsuperscript{15} Egr-1 regulates expression of proinflammatory and procoagulant genes in acute cell stress, and is referred to as a master regulator because it controls expression of as many as 300 genes, many of which are implicated in atherosclerosis, intimal thickening, restenosis, cardiac hypertrophy, and angiogenesis.

Where else might we find SIP and TRPC5 interacting? Possibly in stroke, because the promoter regions for the three proteins also have multiple consensus sequences for the transcription factor, Sp1, which we recently showed undergoes nuclear translocation following cerebral ischemia, where it plays a critical role in death of neural cells.\textsuperscript{16} Moreover, as mentioned above in the context of vascular biology, the promoter regions for the three proteins have consensus sequences for Egr-1, which is induced by hypoxia and is strongly upregulated in cerebral ischemia.\textsuperscript{17,18}

At present, individual involvement of these two ancient families, the sphingolipids and TRP channels, in pathological conditions as diverse as vessel restenosis, atherosclerosis, and stroke remains under active investigation.\textsuperscript{19} However, Beech and colleagues have propelled us down a road that forces us to contemplate not just individual signaling pathways, but rather direct regulatory interactions between the two. Consideration of the parallel transcriptional regulation of specific members of these families lends support to the idea that these systems may be linked in disease, but hard experimental evidence will be needed to prove it. Regardless, it seems reasonable to predict that further study of the interactions between sphingolipid signaling and TRP channels will continue to be fruitful and that other interactions between related members are likely to be uncovered. Most importantly, discovery of such interactions will inevitably reveal new targets for therapy that will hopefully help stem untoward manifestations of disease.

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