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Ceramides – Friend or Foe in Hypoxia?

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Without oxygen, the cells of aerobic animals will eventually die. However, the amount and duration of hypoxia (an abnormally low oxygen environment) required for cell death vary widely in the animal kingdom. Even cells within the same animal can differ greatly in their sensitivity to hypoxia. The ability of an animal to adapt to and survive hypoxia determines fundamental traits such as habitat range and hibernation potential. In humans, cellular injury due to hypoxia causes the most common forms of stroke and heart attack. Cancer cells are often resistant to hypoxic death, and this promotes their tendency to survive after metastasis (1). Thus, the cellular mechanisms that control hypoxic sensitivity are of considerable interest. Most of the known determinants of hypoxic sensitivity have been defined through hypothesis-driven experiments in mammalian cells. By contrast, exploratory genetic strategies that have been so successful at elucidating other complex biological pathways have been relatively underutilized. On page 381 of this issue, Menuz et al. (2) report a serendipitous result from a screen in the worm *Caenorhabditis elegans* for genes controlling anoxic (severely hypoxic) sensitivity.

By testing mutations in candidate genes that might regulate worm sensitivity to anoxia, Menuz et al. discovered a previously unknown set of vicinal mutations in the *hyl-2* gene, which encodes a homolog of the yeast longevity assurance gene, LAG1. LAG1 and LAC1 encode yeast dihydroceramide synthases (3, 4). The amount of LAG1 transcription varies with the replicative age of yeast cells, and deletion of LAG1 increases the life span of haploid yeast cells, suggesting a role for this synthase in cellular aging (5). Expression of wild-type *hyl-2* in yeast cells lacking both LAG1 and LAC1 genes restored yeast viability, indicating that *hyl-2* encodes an authentic ceramide synthase.

Ceramide synthases acylate sphingoid bases with different lengths of fatty acid chains, ranging from 14 to 26 carbon atoms, to produce a family of ceramides (6). Ceramides serve as intermediates for sphingolipids, a major component of cell membranes. Beyond their structural role, ceramides have been implicated as signaling molecules in diverse biological processes in mammalian cells including inflammation, cellular differentiation, and cellular stress responses (7–10). In response to various stresses such as hypoxia and restricted blood supply (ischemia), total cellular ceramide concentration increases, which in turn can activate molecules that induce cell death (apoptosis) (7, 11).

But *C. elegans* has two other ceramide synthase gene homologs, *hyl-1* and *lagr-1*. Both *hyl-1* and *lagr-1* are required for radiation-induced apoptosis, and germline injection of the 16-carbon ceramide can restore germline apoptosis in a *hyl-1* or *lagr-1* deletion mutant (12). Thus, ceramide (at least the 16-carbon ceramide) appears to promote radiation-induced germline apoptosis in *C. elegans*. Unlike worms lacking *hyl-2*, *hyl-1* deletion mutants were more resistant to anoxia than wild-type cells, as might be expected for loss of a proapoptotic gene. Like HYL-2, HYL-1 expression in yeast can rescue the lethal phenotype of a *lag1 lac1* double-deletion mutant (13). Thus, *hyl-1* also encodes an authentic ceramide synthase.

The contrasting anoxic sensitivity phenotypes of the *hyl-1* and *hyl-2* mutants indicate that the two ceramide synthases have distinct functions. By measuring the abundance of ceramide and sphingomyelin species in *hyl-1*(null), *hyl-2*(null), and wild-type worms by mass spectrometry, Menuz et al. determined that *hyl-1* mutant worms contained more 20- to 22-carbon ceramides and sphingomyelin species than wild-type worms, whereas *hyl-2* mutant worms had decreased amounts of these ceramide and sphingomyelin species compared to wild-type worms. Measurement of ceramide synthase activity in isolated microsomes of mutant and wild-type animals confirmed that HYL-1 and HYL-2 have distinct fatty acyl specificities, with *hyl-1* mutant microsomes synthesizing more 20- to 22-carbon ceramides and *hyl-2* mutant microsomes more 24- to 26-carbon ceramides. Together, these data suggest that 20- to 22-carbon ceramide and/or sphingomyelin molecules produced by HYL-2 are protective against anoxic injury. Alternatively, these ceramides and sphingomyelins may not be inherently protective against anoxic injury; rather, their synthesis by HYL-2 in a particular cellular or subcellular context or distribution may be protective.

Given the link between ceramides and apoptosis, Menuz et al. examined the relation between the well-defined apoptosis pathway in *C. elegans* and *hyl-2*. A double-mutant strain carrying a loss-of-function mutation of the apoptosis caspase gene *ced-3* and a *hyl-2* deletion mutation had anoxic sensitivity similar to that of the *hyl-2* deletion mutant strain, indicating that *hyl-2* does not require the canonical apoptosis pathway to control anoxic sensitivity. Menuz et al. also examined the relation between *hyl-2* and *daf-2*. *daf-2* encodes an insulin/insulin-like growth factor receptor homolog that negatively regulates worm life span, stress resistance, and hypoxia resistance (14, 15). The authors found that a *daf-2* reduction-of-function mutant was anoxia resistant. The anoxia resistance of the *daf-2;hyl-2* double mutant was intermediate between that of the two single mutants, which suggests that the two pathways function in parallel to control anoxic sensitivity.

Most of what we know about the function of ceramides in hypoxic and ischemic injury is that they promote cell death in mammals. The findings by Menuz et al. indicate a broader role of ceramides beyond their established proapoptotic one in hypoxic cellular injury. Thus, inhibition of ceramide synthesis is unlikely to be a panacea for hypoxic injury. Rather, development of subtype-specific ceramide synthase inhibitors will likely be necessary. Are ceramide synthases a friend or foe in hypoxic injury? The answer is yes.

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Carbon chain conundrum

In the worm, ceramides with different lengths of fatty acid chains (ranging from 20 to 26 carbons) are produced in response to low oxygen, with different effects on survival. This raises the question of which human ceramide synthases are critical to surviving hypoxic and ischemic injury, such as stroke or heart attack.

Table 1

<i>C. elegans</i>	<u>Ceramide synthases</u>	<u>Ceramides produced</u>	<u>Cell response to anoxia</u>	<u>Cell/organism outcome</u>
	HYL-1	C ₂₄₋₂₆	Sensitivity increases	Death
	HYL-2	C ₂₀₋₂₂	Sensitivity decreases	Survival