Starvation in the midst of plenty: making sense of ceramide-induced autophagy by analysing nutrient transporter expression

Aimee L. Edinger
Department of Developmental and Cell Biology, 2128 Natural Sciences 1, University of California Irvine, Irvine, CA 92697, U.S.A.

Abstract
Ceramide induces differentiation, proliferative arrest, senescence and death in mammalian cells. The mechanism by which ceramide produces these outcomes has proved difficult to define. Building on observations that ceramide stimulates autophagy, we have identified a novel mechanism of action for this sphingolipid: ceramide starves cells to death subsequent to profound nutrient transporter down-regulation. In yeast, ceramide generated in response to heat stress adaptively slows cell growth by down-regulating nutrient permeases. In mammalian cells, a lethal dose of ceramide triggers a bioenergetic crisis by so severely limiting cellular access to extracellular nutrients that autophagy is insufficient to meet the metabolic demands of the cell. In keeping with this bioenergetic explanation for ceramide toxicity, methyl pyruvate, a membrane-permeable nutrient, protects cells from ceramide-induced starvation. Also consistent with this model, we have found that the metabolic state of the cell determines its sensitivity to ceramide. Thus the increased sensitivity of cancer cells to ceramide may relate to their inflexible biosynthetic metabolic programme. These studies highlight the value of assessing nutrient transporter expression in autophagic cells and the important role that culture conditions play in determining the cellular response to ceramide.

Introduction
All cells require transporter proteins to move nutrients across the impermeant plasma membrane. Because these proteins control the cellular fuel supply, modulating nutrient transporter expression is a very effective way to regulate cell growth and survival [1]. For example, growth factors promote cell growth and survival in part by increasing nutrient transporter surface levels [1–4]. Conversely, the down-regulation of nutrient-transporter proteins following growth factor withdrawal plays a critical role in the induction of apoptosis [5,6]. When transporter turnover is blocked, cells become partially transformed and capable of growth-factor-independent cell survival [5]. Nutrient transporter turnover limits cell growth even in cells unable to undergo apoptosis. For example, cells lacking the pro-apoptotic proteins Bax and Bak can survive growth factor withdrawal for over 6 weeks [7]. However, these cells atrophy continuously and become dependent upon autophagy owing to the loss of nutrient-transporter proteins. In the end, even Bak/Bax double-knockout cells starve to death in the absence of growth factors because catabolism is a self-limiting survival strategy. Taken together, these studies demonstrate that growth factors regulate both cell growth and survival by controlling nutrient uptake (Figure 1).

Because nutrient transporter down-regulation leads to intracellular nutrient limitation, autophagy is induced in growth-factor-deprived cells. Autophagy is an evolutionarily conserved response to starvation by which cells catabolize their components to create an internal supply of essential nutrients [8]. Constitutive autophagy also promotes cellular health by facilitating the turnover of damaged or unnecessary organelles. Autophagy is induced under a wide variety of conditions and, in some cases, contributes to cell death. Now that essential mammalian autophagy genes have been identified, it is possible to discriminate between ‘good’ and ‘bad’ autophagy by specifically inhibiting this process using RNAi (RNA interference) or gene-deletion strategies. If cell survival increases when autophagy is blocked, autophagy is likely to contribute to cell death. If viability declines when autophagy is prevented, autophagy is classified as an adaptive response. Experiments discriminating between helpful and harmful autophagy have dramatically increased our understanding of the role autophagy plays in the aetiology of multiple human diseases. However, in the event that autophagy is found to be protective, there is an obvious next question that is seldom addressed: is the autophagic cell starving, or not? Because many of these studies have been carried out in cell culture systems where nutrients are virtually unlimited, the possibility that cells could be starving may have been discounted. However, it is intracellular, not extracellular, nutrient levels that determine whether a cell is nutrient-limited. Insufficient import of abundant extracellular nutrients might well account for much of the homoeostatic autophagy that is observed in rich media. Similarly, although homoeostatic feedback loops maintain nutrient levels in the bloodstream within a narrow range, even during periods of...
Growth factor withdrawal produces intracellular nutrient limitation secondary to nutrient transporter down-regulation

In the presence of growth factors (orange diamond), nutrient-transporter proteins are expressed on the cell surface. Nutrients enter the cell via these transporters, providing substrates for ATP generation via glycolysis in the cytosol and the TCA cycle in the mitochondria. In the presence of growth factors, cells run a metabolic programme that uses glycolytic and TCA cycle intermediates in biosynthetic reactions. ATP is also consumed in transcription and translation. Few autophagosomes (green cup-shaped structure) form as nutrients are derived from extracellular sources. When growth factors become limiting, nutrient-transporter proteins are internalized and degraded. Metabolism becomes substrate-limited, and cells switch from a biosynthetic to a catabolic metabolic programme. Autophagy is induced to support essential cellular processes using internally derived nutrients.

reduced food intake, cells in vivo may also induce autophagy as a starvation response if nutrient-transporter proteins have been down-regulated. Our recent studies in ceramide-treated cells highlight the value of considering autophagy in the context of nutrient transporter expression.

Ceramide negatively regulates nutrient transporter expression

Ceramide limits growth, proliferation and survival, and promotes differentiation and senescence in mammalian cells [9]. It is therefore not surprising that deregulation of ceramide metabolism has been linked to numerous human diseases. Ceramide plays a particularly well-defined role in cancer: ceramide has been called a ‘tumour-suppressor lipid’ based on its ability to block tumour initiation and metastasis [10]. Consistent with the fact that many cancer chemotherapeutics work by causing ceramide generation, ceramide is more toxic to tumour cells than to normal cells. Ceramide can directly activate several kinases and phosphatases, is a component of lipid rafts and has poorly defined effects on membranes that may include the formation of channels [10,11]. How these or other ceramide-induced changes would lead to the selective demise of tumour cells is uncertain. Studies showing that ceramide triggers autophagy and older studies performed in yeast have, however, provided important clues.

Yeast produce sphingolipids closely related to ceramide as a part of the heat-stress response [12,13]. These sphingolipids adaptively slow cell growth by down-regulating a variety of nutrient permeases: substrate-limitation prevents cell growth under these dangerous conditions where many proteins would probably misfold. In the light of the fact that ceramide induces autophagy in mammalian cells [14–16], we proposed that ceramide might also down-regulate nutrient-transporter proteins in mammalian cells. To test this idea, we measured nutrient transporter surface expression in cells exposed to cell-permeable short-chain ceramide [17]. Physiologically relevant concentrations of C2-ceramide produced a rapid and profound down-regulation of amino acid and glucose transporters in multiple cell types. Autophagy followed nutrient transporter loss, but both processes occurred before the initiation of apoptosis. Consistent with the idea that ceramide-exposed cells were nutrient-limited, blocking autophagy increased sensitivity to ceramide, whereas blocking transporter internalization increased cell viability. If ceramide-treated cells starve to death because they have lost access to extracellular nutrients subsequent to nutrient transporter down-regulation (Figure 2A), then a membrane-permeable transporter-independent nutrient should protect cells from ceramide. Unlike sodium pyruvate, methyl pyruvate can cross the plasma membrane without a transporter. Pyruvate, the end product of glycolysis, can fuel the TCA (tricarboxylic acid) cycle and allow the synthesis of a variety of amino acids, nucleotides and lipids either directly or by means of TCA cycle intermediates. In keeping with a model where the loss of access to extracellular nutrients is responsible for the death of ceramide-exposed cells, methyl pyruvate supplementation both protected cells from ceramide-induced death and delayed the induction of autophagy (Figure 2B).

A word of caution to those who would use rescue by methyl pyruvate as a test of whether a cell is undergoing autophagy secondary to bioenergetic stress: methyl pyruvate does not rescue all cell types equally well, and is even
Ceramide produces intracellular nutrient limitation secondary to nutrient transporter down-regulation

(A) Similarly to growth-factor-withdrawn cells (Figure 1), cells exposed to ceramide also down-regulate nutrient-transporter proteins and switch from a biosynthetic to catabolic metabolic programme. (B) Because it can cross the plasma membrane without a transporter, methyl pyruvate rescues ceramide-exposed cells. Consistent with the proposal that it functions as a cell-permeable nutrient, methyl pyruvate slows the induction of autophagy.

Figure 2 | Ceramide produces intracellular nutrient limitation secondary to nutrient transporter down-regulation

(A) Similarly to growth-factor-withdrawn cells (Figure 1), cells exposed to ceramide also down-regulate nutrient-transporter proteins and switch from a biosynthetic to catabolic metabolic programme. (B) Because it can cross the plasma membrane without a transporter, methyl pyruvate rescues ceramide-exposed cells. Consistent with the proposal that it functions as a cell-permeable nutrient, methyl pyruvate slows the induction of autophagy.

toxic in some cases ([17] and A.L. Edinger, K.R. Rosales and G. Guenther, unpublished work). The variable response of different cell types to methyl pyruvate may relate to differences in basal metabolism that render cells more or less able to productively utilize the extra pyruvate to support essential functions. For example, for methyl pyruvate to serve as the sole fuel source for the TCA cycle, cells must be able to convert it into both oxaloacetate and acetyl-CoA; this may not be possible in all cells under all conditions. If methyl pyruvate is provided in excess of the amount that can enter the TCA cycle or be otherwise utilized in biosynthetic reactions, the extra pyruvate may be converted into lactate, creating acidosis. Furthermore, the absence or scarcity of other nutrients would limit the ability to use methyl pyruvate as a building block in biosynthetic reactions (e.g. the production of amino acids or nucleotides). The bottom line is that the metabolic pathways that are active in the supplemented cell and the availability of other molecules will dramatically influence whether methyl pyruvate can correct a bioenergetic defect. At best, methyl pyruvate is life support for starving cells, not a miracle nutrient that can ‘cure’ bioenergetic stress.

Implications of ceramide-induced nutrient transporter down-regulation for tumour initiation and progression

Our model suggesting that ceramide has a bioenergetic mechanism of action may also explain the selectivity of ceramide and chemotherapeutics that cause ceramide generation such as daunorubicin and etoposide for cancer cells. The biochemical re-programming that occurs in cancer cells primes them for a metabolic disaster should fuel supplies run low [18–20]. For example, the tumour suppressor p53 is deleted in the majority of human tumours. Although it prevents cell death and blocks senescence in tumour cells, loss of p53 also increases cellular sensitivity to glucose withdrawal by blocking the AMPK (AMP-activated protein kinase)-dependent cell-cycle arrest that is part of the adaptive response to glucose depletion.
Metabolic changes that promote oncogenesis make cancer cells more sensitive to ceramide

Cancer cells express constitutively active oncogenes that drive biosynthetic reactions and suppress catabolic processes such as autophagy. In addition, transformed cells have deleted tumour-suppressor proteins that facilitate quiescence. Thus cancer cells have eliminated the proteins that help cells to adapt to ceramide-induced transporter loss and have activated proteins that will exacerbate intracellular nutrient limitation following nutrient transporter down-regulation. Whereas normal cells become quiescent in response to non-lethal dose of ceramide, the same level of ceramide causes a bioenergetic crisis in cancer cells that leads to cell death.

21. p53 also controls the fate of glucose-derived carbon via TIGAR (TP53 tumour protein 53)-induced glycolysis and apoptosis regulator [22] and the rate of mitochondrial respiration via the copper transporter SCO2 that participates in the assembly of cytochrome c oxidase (complex IV in the respiratory chain) [23]. The metabolic changes orchestrated by p53, and its ability to promote autophagy via DRAM (damage-regulated autophagy modulator) [24], would help normal cells adapt to bioenergetic stress. Not only have cancer cells inactivated tumour-suppressor proteins such as p53, they also activate oncogenes that drive growth by promoting biosynthetic metabolism. Akt, a kinase activated in many human tumours, stimulates glycolysis and increases protein and lipid synthesis while blocking β-oxidation and autophagy (reviewed in [18]). These metabolic changes make cells with constitutively active Akt exquisitely sensitive to glucose depletion [25]. Because tumour cells cannot turn off biosynthetic programmes and are unable to activate quiescence and catabolic programmes, an increase in ceramide that produces cell-cycle arrest in normal cells is likely to induce a bioenergetic catastrophe in cancer cells (Figure 3).

This model will, of course, require experimental validation. However, several pieces of available evidence strengthen this argument. Cells maintained in high levels of growth factors are more, not less, sensitive to ceramide [17]. This result contradicts expectations based on the ability of growth factors to block apoptosis, but is in perfect accord with the effects of growth factors on bioenergetics. High levels of growth factors drive glycolysis and biosynthesis, whereas low levels of growth factors are only sufficient to support a conservative, more quiescent, metabolic programme [26,27]. The contrary effects of acute and gradual nutrient restriction are also consistent with the metabolic explanation for ceramide’s enhanced toxicity to transformed cells. Acute nutrient restriction sensitizes cells to ceramide; the decrease in substrate availability increases the impact of having fewer nutrient-transporter proteins at the cell surface [17]. On the other hand, adapting cells gradually to low nutrient levels produces cells that are completely insensitive to ceramide. The protection afforded by growing cells in low nutrients also helps to rule out the possibility that ceramide kills cells through non-specific ‘membrane effects’.

A crucial step on the path to transformation is disabling apoptosis [28]. Thus cancer therapies must be able to eliminate cells that are resistant to this form of cell death. Consistent with the clinical success of the many chemotherapeutics that depend on ceramide generation, we found that blocking apoptosis delayed, but did not prevent, ceramide-induced death [17]. Apoptosis-deficient cells exposed to ceramide quickly became riddled with autophagic vacuoles reminiscent of the dramatic increase in autophagy observed in growth-factor-withdrawn cells deficient in the pro-apoptotic proteins Bak and Bax.
and Bax [7]. As would be expected in cells that are nutrient-limited, blocking apoptosis leads to caspase-independent cell death in ceramide-treated cells [17]. This non-apoptotic death was also blocked by methyl pyruvate, confirming its bioenergetic origin. These findings are consistent with observations by other groups that ceramide can produce necrosis [29].

These studies suggest several new approaches to cancer therapy. Because resistance to many chemotherapeutics is conferred by mutations that decrease ceramide generation [30–32], drugs that stimulate nutrient transporter down-regulation directly may bypass the need for ceramide production and kill multidrug-resistant tumour cells. Our finding that autophagy protects cells from ceramide suggests further that chemotherapies that induce ceramide production might work synergistically with drugs that block autophagy. It has already been demonstrated that chloroquine, a chemical inhibitor of autophagy, decreases the rate of EBV (Epstein–Barr virus)-induced lymphoma in humans and delays the progression of Myc-overexpressing tumours in mice [33,34]. Chloroquine is a relatively safe drug with few side effects, and it will be very interesting to determine whether chloroquine increases the efficacy of ceramide-generating chemotherapeutics.

Ceramide, transporters and Type 2 diabetes

Type 2 diabetes is associated with obesity and characterized by insulin resistance; insulin is present in the bloodstream, at least in the initial stages of the disease, but blood glucose levels remain high as the target cells fail to respond appropriately to insulin stimulation. The pathogenesis of Type 2 diabetes is not completely understood, but ceramide generation appears to play a role [35]. Increased dietary fat intake is thought to lead to increased serum palmitate which is converted into ceramide via the de novo ceramide synthetic pathway. Blocking ceramide generation via the de novo pathway prevented the development of diabetes in mice on a diabetogenic diet [36]. Precisely how ceramide generation leads to Type 2 diabetes in these mice or in humans is unclear. Our work suggests that ceramide might contribute to the aetiology of this disease by down-regulating glucose transporters, thereby blocking insulin action.

Concluding remarks

Having established that nutrient transporter down-regulation plays a central role in ceramide-induced cell death, an important next step will be to identify the molecules that coordinate nutrient transporter down-regulation in response to ceramide as these proteins represent important therapeutic targets. At present, virtually nothing is known about how nutrient transporter internalization and trafficking is regulated in mammalian cells. The existence of global regulators of nutrient transporter expression in yeast suggests that orthologous proteins will eventually be found in mammalian cells [37]. Given that yeast ceramides regulate not just nutrient permease turnover, but also senescence and lifespan [38], it is also possible that drugs that limit nutrient transporter expression would slow the mammalian aging process through the same mechanisms as extracellular nutrient limitation (caloric restriction) [39]. Additional studies will be required to evaluate these highly speculative but intriguing proposals.

References


Acknowledgements

I thank Ralph DeBerardinis for valuable conversations regarding ceramide metabolism and cellular bioenergetics.

Funding

Supported by National Institutes of Health [grant number K08 CA100526] and a grant from Gabrielle’s Angel Foundation for Cancer Research [grant number 034].


Received 30 September 2008
doi:10.1042/BST0370253