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Commentary

Recent intense interest has focused on the activation of the mitochondrial pathway to apoptosis, particularly as it occurs in tumor cells treated with cytotoxic agents such as doxorubicin and etoposide. The contribution of two prominent molecular players in this response, the sphingolipid second messenger ceramide and the novel protein kinase C isoform (PKC δ), are only now being appreciated, but their significance and their interactions with one another remain controversial. While some studies argue strongly that ceramide generation is critical to apoptosis induced by certain drugs (1, 2), others have cast doubt on this notion (3, 4). A variety of stress stimuli, as well as radiation and anti-cancer drugs, generate ceramide through the sphingomyelinase (SMase) pathway (Figure 1). SMases are analogous in their action to phospholipases C, but act specifically on the ubiquitous membrane phospholipid sphingomyelin. Neutral and acidic forms of SMase (distinguishable by their pH optima) have been described, and both are implicated in the induction of apoptosis (5, 6). Another, de novo route to ceramide biosynthesis may also be involved in this process (1, 2, 7). Here, serine palmitoyl-transferase catalyzes the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine, which is reduced to sphinganine and subsequently N-acylated by ceramide synthase to form dihydroceramide. Finally, a double bond is introduced by dihydroceramide desaturase to produce ceramide (N-acyl sphingosine). However it [...]

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Recent intense interest has focused on the activation of the mitochondrial pathway to apoptosis, particularly as it occurs in tumor cells treated with cytotoxic agents such as doxorubicin and etoposide. The contribution of two prominent molecular players in this response, the sphingolipid second messenger ceramide and the novel protein kinase C isoform (PKC δ), are only now being appreciated, but their significance and their interactions with one another remain controversial. While some studies argue strongly that ceramide generation is critical to apoptosis induced by certain drugs (1, 2), others have cast doubt on this notion (3, 4).

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1). SMases are analogous in their action to phospholipases C, but act specifically on the ubiquitous membrane phospholipid sphingomyelin. Neutral and acidic forms of SMase (distinguishable by their pH optima) have been described, and both are implicated in the induction of apoptosis (5, 6). Another, *de novo* route to ceramide biosynthesis may also be involved in this process (1, 2, 7). Here, serine palmitoyl-transferase catalyzes the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine, which is reduced to sphinganine and subsequently *N*-acylated by ceramide synthase to form dihydroceramide. Finally, a double bond is introduced by dihydroceramide desaturase to produce ceramide (*N*-acyl sphingosine). However it is formed, accumulating ceramide apparently activates cytochrome *c* release, caspase activation,

and apoptosis (5, 6); the direct targets of ceramide in this pathway are still a matter of debate.

Many groups have also demonstrated that induction of apoptosis by cytotoxic drugs or by phorbol esters is associated with translocation of PKC δ to the mitochondria, accompanied by cytochrome *c* release and activation of the apoptotic cascade (8–11). In some systems, interference with PKC δ translocation/activation has been shown to attenuate drug-induced lethality (8–10). Again, however, the functional significance of these events in the apoptotic process remains to be fully defined (12).

The relationship between ceramide and PKC δ is highly complex, with evidence that ceramide generation can represent either a cause (13, 14) or a consequence (7) of PKC δ activation. Efforts to reconcile these disparate

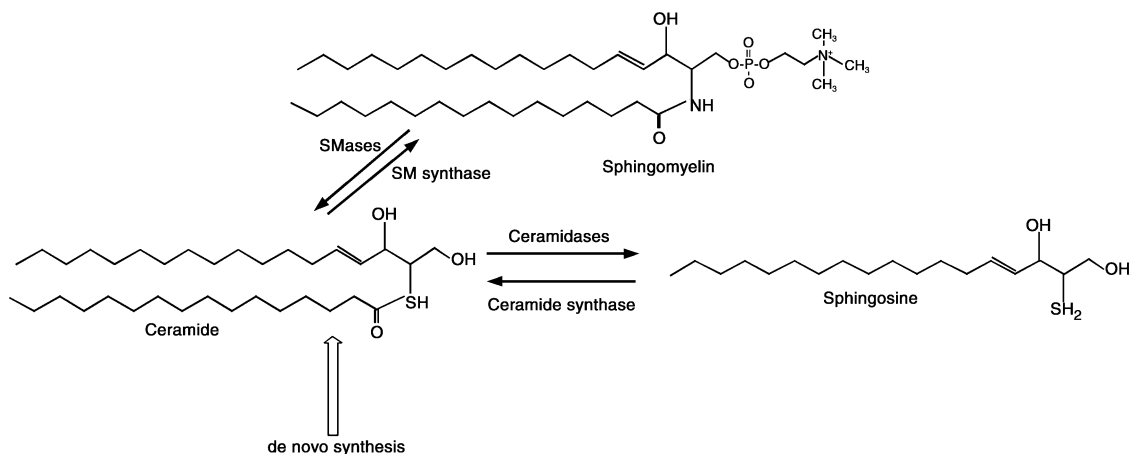


Figure 1

Formation of ceramide in mammalian cells. Many stress stimuli, including anticancer drugs, γ -radiation, ultraviolet, cytokines, and growth factor withdrawal, activate sphingomyelinases, which remove the head group from the membrane phospholipid sphingomyelin, forming ceramide. An opposing reaction, catalyzed by sphingomyelin synthase, occurs when phosphocholine is transferred from phosphatidylcholine to the primary hydroxyl of ceramide, producing sphingomyelin and diacylglycerol. In addition, ceramide can be produced through a *de novo* biosynthetic pathway, described in the text. Sphingosine is not produced by the *de novo* pathway and can only be formed by deacylation of ceramide catalyzed by ceramidases.

findings have been hampered by the lack of a unified model system in which to define the interactions between ceramide and PKC δ and explore their roles in regulating cell death. In a report appearing in this issue of the *JCI*, Sumitomo and colleagues provide a conceptual model that might help to clarify these issues (15). These authors show that in LNCaP prostate cancer cells, which express PKC δ , exposure to cytotoxic drugs such as etoposide and paclitaxel induces a biphasic increase in ceramide synthesis. In the initial phase, transient increases in ceramide levels stem from stimulation of the de novo synthetic pathway. Subsequent, sustained increases in ceramide levels, however, depend on neutral sphingomyelinase activation and sphingomyelin hydrolysis. Significantly, the latter process appears to be dependent upon caspase-9 activation, which in turn represents a consequence of translocation of PKC δ to the mitochondria and release of the proapoptogenic protein cytochrome *c*. In this way, the generation of ceramide and the translocation of PKC δ to the mitochondria — resulting from drug treatment — cooperate to amplify apoptotic signals, thereby ensuring complete activation of the cell death cascade.

Aside from providing insights into the mechanism by which cytotoxic drugs induce prostate cancer cell death, these observations could also help to reconcile conflicting findings in literature regarding the hierarchical roles of ceramide and PKC δ in apoptotic events. For example, the finding of an initial, transient increase in de novo ceramide synthesis in drug-treated cells is entirely compatible with the notion that upstream-generated ceramide is responsible for PKC δ translocation (13, 14). On the other hand, the evidence that drug-induced PKC δ translocation and activation plays a functional role in the caspase-9-mediated generation of ceramide and potentiation of apoptosis is consistent with reports indicating that activation of PKC δ by phorbol esters in LNCaP prostate cancer cells contributes to ceramide formation (7). The complexity of this feedback amplification loop is underscored by the observation that caspase-9 inhibition also attenuates

PKC δ mitochondrial translocation, implying that PKC δ can promote its own activation through a ceramide-mediated process. Similarly, induction of apoptosis by cross-linking of Fas triggers the biphasic generation of ceramide, which is then converted to sphingosine. Along with ceramide and released cytochrome *c*, sphingosine itself can activate caspase-8 and the proapoptotic Bcl-2 family member, Bid. Other proapoptotic molecules, perhaps the mitochondrion-derived, apoptosis-inducing factor (AIF), may be required to amplify this process (16). This would not be the first example of an amplification loop sealing the fate of tumor cells following cytotoxic drug exposure. Drug-induced cytochrome *c* release can result in caspase-3 activation, leading in turn to the secondary cleavage of pro-caspase-8, Bid activation and mitochondrial translocation, and further mitochondrial injury (17).

A number of questions remain to be answered before the role of ceramide and PKC δ in apoptosis can be more fully understood. For instance, recent studies suggest that Bcl-2 and Bcl-xL act by disrupting interactions between Bcl-2 homology domain-3 (BH3), domain-only family members and multidomain pro-apoptotic proteins such as Bax and Bak to trigger cytochrome *c* release (18). Questions therefore arise as to how, whether, and at what level ceramide and PKC δ might interact with these proteins to promote mitochondrial injury and initiation of the apoptotic caspase cascade. How does ceramide, synthesized in the endoplasmic reticulum, regulate translocation of PKC δ to the mitochondria? There is some evidence suggesting that ceramide might bind directly to PKC δ (19), but it is also possible that the ability of ceramide to form platforms of sphingolipid-enriched membrane domains (20) is involved in translocation of PKC δ to the mitochondria.

Another important question concerns the identity of the neutral SMases activated by caspase-9, as the relevant molecule has not yet been cloned. In addition, even if ceramide and PKC δ can be shown to play key roles in drug-induced apoptosis in certain cells, other forms of non-apoptotic cell death have been

described. For this reason, cytotoxic drugs might inhibit self-renewal even in tumor cells defective in ceramide- and/or PKC δ -dependent apoptosis. A final consideration relates to the effects of ceramide generation and PKC δ activation on other pro-apoptotic mitochondrial proteins, including AIF (21) and Smac/DIABLO, which antagonize the actions of inhibitor of apoptosis proteins (22). In this context, it is interesting that a recent study (23) suggests that release of Smac/DIABLO is required for activation of the caspase cascade by cytochrome *c* in prostate cancer cells. Answers to these questions are greatly needed to deepen our understanding of apoptosis and to inspire new approaches for treating prostate cancer or other malignancies.

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