Hypothesis

Apoptosis and signalling in acid sphingomyelinase deficient cells

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Abstract

Background: Recent evidence suggests that the activation of a non-specific lipid scramblase during apoptosis induces the flipping of sphingomyelin from the cell surface to the cytoplasmic leaflet of the plasma membrane. Inner leaflet sphingomyelin is then cleaved to ceramide by a neutral sphingomyelinase. The production of this non-membrane forming lipid induces blebbing of the plasma membrane to aid rapid engulfment by professional phagocytes. However contrary evidence suggests that cells which are deficient in acid sphingomyelinase are defective in apoptosis signalling. This data has been interpreted as support for the activation of acid sphingomyelinase as an early signal in apoptosis.

Hypothesis: An alternative explanation is put forward whereby the accumulation of intracellular sphingomyelin in sphingomyelinase deficient cells leads to the formation of intracellular rafts which lead to the sequestration of important signalling molecules that are normally present on the cell surface where they perform their function.

Testing the hypothesis: It is expected that the subcellular distribution of important signalling molecules is altered in acid sphingomyelinase deficient cells, leading to their sequestration in late endosomes / lysosomes. Other sphingolipid storage diseases such as Niemann-Pick type C which have normal acid sphingomyelinase activity would also be expected to show the same phenotype.

Implications of the hypothesis: If true the hypothesis would provide a mechanism for the pathology of the sphingolipid storage diseases at the cellular level and also have implications for the role of ceramide in apoptosis.

Background

Recently reported data shed further light on the interrelationships between caspase activation, the scrambling of membrane phospholipid asymmetry and the production of ceramide which can occur during apoptosis signalling. Firstly, since signalling by the caspase cascade can occur very rapidly elucidation of whether ceramide production or caspase activation comes first is of great importance. A large body of evidence now suggests that inhibition of inducer caspases such as FLICE also inhibit ceramide formation [1–3]. These observations suggest that ceramide generation is downstream of the early signalling events in apoptosis. Moreover, late generation of ceramide indicates that ceramide formation may be a consequence of the execution of apoptosis rather than a signal. During the execution of apoptosis the activation of a non-specific phospholipid membrane scramblase leads to the general disruption of phospholipid asymmetry, the externalisation of phosphatidylserine and membrane blebbing. Membrane blebbing and
phosphatidyserine exposure signal a physiological end-point since they lead to engulfment by professional phagocytes and rapid clearance from the tissue.

As early as the 1980’s it was suggested that ceramide production and membrane blebbing are linked [4]. Allan and co-workers reported that treatment of chicken erythrocytes with Ca^{2+} ionophore A23187 led to a loss of membrane phospholipid asymmetry and membrane blebbing presumably through the activation of a non-specific phospholipid scramblase. Scrambling of membrane asymmetry was associated with increases in ceramide by the action of an intracellular sphingomyelinase. More recently, van Blitterswijk and co-workers have found that activation of a non-specific phospholipid scramblase during Fas-mediated apoptosis is responsible for the generation of ceramide [5]. They show that this ceramide is derived from cell surface sphingomyelin and is cleaved due to flipping of external sphingomyelin towards the inner leaflet of the plasma membrane by an intracellular sphingomyelinase. Simultaneously, inner leaflet phosphatidylserine is flipped to the cell surface. Thus ceramide generation may be a consequence of intercellular signalling for phagocytosis of apoptotic cells. Breakdown of sphingomyelin and the production of ceramide may be very important for the changes in cell morphology that occur during apoptosis. In contrast to sphingomyelin, ceramide is a hydrophobic lipid without a polar headgroup that does not form membrane bilayers in aqueous environments. Ceramide formed from the hydrolysis of sphingomyelin is expected to accumulate in the membrane interior and lead to membrane blebbing. Simultaneously, removal of cell surface sphingomyelin also has a destabilising effect since sphingolipids form complexes (rafts) with cholesterol. This is due to hydrogen bonding between the hydroxyl group of cholesterol and the hydroxyl group of the sphingosine backbone as well as hydrophobic van der waals interactions with the saturated acyl chains that tend to be enriched in sphingolipids. These changes would be expected to be important in facilitating the changes in membrane curvature that occur during blebbing allowing rapid phagocytosis and by-passing the potentially damaging inflammation that occurs during necrotic cell death. However, recent evidence that acid sphingomyelinase deficient cells have defects in apoptotic signalling pathways have been interpreted as strong evidence for the role of ceramide in signalling. This is despite disparate results with acid sphingomyelinase deficient cells [6]. Indeed it has been observed that splenocytes derived from a NPD mouse in which the acid sphingomyelinase gene has been knocked out can show enhanced apoptosis at advanced stages of the disease [7]. Still it has been claimed that these paradoxical results are due to abnormally large levels of sphingomyelin accumulation in older mice and that young mice that do not store large amounts of sphingomyelin are defective in apoptosis [8]. These results have been interpreted to support the activation of a sphingomyelinase in a signalling cascade that is an important initial event in apoptosis ie before the activation of inducer caspas [9–11]. In order to clarify some of these issues the following hypothesis is proposed:

Presentation of the hypothesis

An alternative explanation for the observed defects in apoptosis signalling in acid sphingomyelinase deficient cells

It is hypothesised that changes in apoptosis signalling in these cells are due to indirect effects of decreased sphingomyelin breakdown rather than the inhibition of ceramide formation. Recent evidence suggests that sphingolipid-enriched rafts form sorting platforms for specific proteins and lipids in the endocytic pathway perhaps especially in the endocytic sorting of specific membrane components to the Golgi apparatus [12,13]. Lysosomal accumulation of sphingomyelin is expected to disrupt endocytic trafficking of important raft-associated cell surface signalling molecules. Sphingomyelin is usually rapidly broken down in the late endosomes and lysosomes. In acid sphingomyelinase deficient cells sphingomyelin can be kinetically trapped in this organelle even in young mice which do not store large amounts of sphingomyelin. Through its association with cholesterol this leads to the formation of rafts in the late endosomes and disruption of the normal trafficking of raft associated proteins and lipids.

Testing the hypothesis

Defects in apoptosis signalling would be expected to occur in other sphingolipid storage disorders, such as glycosphingolipid storage disorders and Niemann–Pick type C which are not defective in sphingomyelinase. Cells storing large amounts of sphingolipid should show changes in the subcellular location of signalling molecules, especially those which are associated with rafts [14].

Implications of the hypothesis

The accumulation of sphingomyelin in the lysosome of these cells may have diverse consequences for the cell’s biology including the increased localisation of raft-associated proteins which normally cycle through the early endocytic pathway and Golgi apparatus with the lysosome [12,13]. Such an effect may be expected to lead to reduced surface expression of raft-associated receptors and their effectors. Inhibition of acid sphingomyelinase induces a lipid traffic jam and may lead to the sequestration and consequent inactivation and breakdown of raft-associated proteins in the lysosome. Such events may contribute indirectly to decreased apoptotic signalling.
If true, the evidence suggests a role for ceramide in the blebbing of apoptotic cells in the execution phase of apoptosis to aid their rapid clearance by professional phagocytes. This role is in contrast to the structurally related lipid, diacylglycerol, which serves as a second messenger in many agonist stimulated events.

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