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Addenda

Is Autophagy the Key Mechanism by Which the Sphingolipid Rheostat Controls the Cell Fate Decision?

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ABSTRACT

Sphingolipids are major constituents of biological membrane and some of them behave as second messengers involved in the cell fate decision. Ceramide and sphingosine 1-phosphate (S1P) constitute a rheostat system in which ceramide promotes cell death and S1P increases cell survival. We have shown that both sphingolipids are able to trigger autophagy with opposing outcomes on cell survival. Here we discuss and speculate on the diverging functions of the autophagic pathways induced by ceramide and S1P, respectively.

CERAMIDE AND S1P BOTH TRIGGER AUTOPHAGY

The metabolism of sphingolipids is a dynamic process generating second messengers such as ceramide and S1P.1 The formation of ceramide is followed by a deacylation to form sphingosine, which is subsequently phosphorylated by sphingosine kinases (SK), leading to S1P formation. It is now generally accepted that ceramide and S1P have contrasting roles on the response to cell stress.2 Ceramide is associated with cell growth arrest and cell death induction, whereas S1P stimulates proliferation and maintains survival in numerous cell types.

Our group has investigated the correlation between sphingolipid metabolism, the autophagic capacities and the cell signaling pathways; we showed that ceramide and S1P are able to induce autophagy in a breast cancer cell line.3,4

First, we showed that ceramide could mediate tamoxifen-induced autophagy, which represents a model of autophagic cell death.5 It was shown that tamoxifen induced an increase of endogenous ceramide levels responsible for a robust accumulation of Beclin1 and an inhibition of Akt/PKB phosphor ylation, which tend to increase the autophagic response. In addition, we also showed that treatment by short permeant ceramide (C2-ceramide) mimicked these effects. Accordingly, pharmacological inhibition of endogenous long chain ceramide biosynthesis by fumonisin B1 (FB1), prevents autophagic induction by tamoxifen. Interestingly, Kondo and colleagues have shown, that treatment of glioma cells with C2-ceramide increased autophagy and cell death by activating Bnip3, a proapoptotic mitochondrial protein.6 These modulatory effects on protein expression appear to constitute three alternative steps for ceramide to regulate autophagy.

The fact that ceramide and S1P constitute a rheostat system involved in the control of cell death, prompted us to investigate the role of S1P on autophagy. Surprisingly, we observed that the increase of S1P levels after sphingosine kinase 1 overexpression is able to induce autophagy.3 This S1P-induced autophagy correlates with the inhibition of the mTOR activity (without alteration of Akt/PKB phosphorylation) and a moderate increase in the amount of Beclin 1. In addition, SK1 activity increased during nutrient starvation, and inhibition of SK1 by siRNA prevented autophagy and exacerbated cell death with apoptotic hallmarks induced by starvation. These results suggest that autophagy is a novel function of S1P in cell survival.

TWO DIFFERENT AUTOPHAGIC PROCESSES CONTROLLED BY SPHINGOLIPIDS?

The above observations reinforced the ambiguous involvement of autophagy in the cell lifetime. However, some key differences were noted between ceramide and S1P-induced autophagy that could account for the decision of the cell to survive or die. Distinctions appear in the step of signaling pathway alteration (ceramide acts upstream of S1P), in the amplitude of the autophagic response (ceramide could trigger a stronger response) and in

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the nature of the stress (only SK activity increased after starvation) (Fig. 1).

Ceramide acts upstream of S1P by inhibiting Akt/PKB phosphorylation, which is associated with autophagy suppression and reduction of cell viability.\(^7,8\) S1P acts only at the level of mTOR, independently of the PI3K arm, in a direct or indirect manner, as suggested by the inhibition of phosphorylation of mTOR substrates (p70S6K, 4E-BP1). Does this observation mean that in comparison with S1P, ceramide has a broad-spectrum of targets potentially linked to cell death induction.

Although Beclin 1 is a common target for both sphingolipids, a major difference appears. Only ceramide induces a strong accumulation of Beclin 1 (2.5 fold higher than after S1P enhancement, according to Fig. 6 of Lavieu et al.\(^3\)). Because the interaction between Bcl-2 and Beclin 1 is required to maintain autophagy in a physiological range,\(^9\) it is tempting to speculate that the strong increase of Beclin 1 induced by ceramide changes the ratio of Beclin 1/Bcl-2 in a range incompatible with survival. Moreover, it was shown by Bektas et al.\(^10\) that the increase of SK activity is tightly associated with the enhancement of Bcl-2 expression. This observation, in addition to the moderate accumulation of Beclin 1 after SK1 overexpression, seems to be compatible with the Bcl-2/Beclin 1 rheostat hypothesis. Does it mean that the autophagic response triggered by S1P is gentler than ceramide-induced autophagy and compatible with cell survival? Interestingly, a fine analysis of the data from Lavieu et al.\(^3\) (see Fig. 2), tends to support this suggestion, even though the method used to induce autophagy by the two lipids is different (treatment by exogenous short-chain ceramide for ceramide-induced autophagy vs. enforced expression of SK1 for the S1P-induced autophagy). The GFP-LC3 dot formation is lower in the case of S1P-induced autophagy (approximately 1.6 fold lower than after C2-ceramide treatment). The ceramide response seems to be also faster than the S1P response (4 hours vs. at least one day).

However, this point would deserve further study by analyzing the effect of ceramide and S1P on the interaction between Bcl-2 and Beclin 1.

One of the major differences between the two responses is the nature of the stress situation, since only S1P-induced autophagy occurs after nutrient starvation. We have shown that starvation induced both autophagy and an increase of SK activity. This last point was equally observed in yeast suggesting a general role for SK during the starvation response.\(^11\) We have shown that the knock-down of SK1 expression by siRNA inhibits starvation-induced autophagy and increased cell death. For this reason we proposed that S1P is a mediator of starvation-induced autophagy which is now well established as a survival mechanism.\(^12\)

Surprisingly, the amount of endogenous ceramide during starvation remained unchanged (from 15 min to 48 h) and the autophagic response after inhibition of ceramide biosynthesis by FB1 treatment was unaffected. These results suggest that ceramide is excluded from the regulation of starvation-induced autophagy. Does it mean that ceramide-induced autophagy and S1P-induced autophagy are mutually exclusive responses? Study of enzymes that regulate the balance between ceramide and S1P could be one of the ways to resolve these questions.

While the above discussion deals with sphingolipids as second messengers and their role in signal transduction, we should not forget that these molecules are constituents of biological membranes and that, because of their biophysical properties, modulate membrane functions. For example it is now established that ceramide is responsible for a negative curvature of the membrane.\(^13\) Moreover, it was shown that alteration of ceramide biosynthesis results in changes of the vacuole morphology in the yeast *Saccharomyces cerevisiae*.\(^14\) Additionally, studies have described a change in SK1 localization, which is usually cytosolic. For example, SK1 is recruited to the nascent phagosome in human macrophages during the phagocytosis of *mycobacterium tuberculosis*.\(^15\) What is the significance of this enzyme delocalization? Where is localized SK1 during autophagy? It is an attractive possibility to contemplate that, in addition to its signaling activity, ceramide and other lipids could play a structural role in the autophagosome biogenesis. Progress in microscopy and the development of fluorescent probes for sphingolipid trafficking analysis need to be considered in order to identify the origin of the autophagosome, an old and still unanswered question.\(^16\)
References