

20. Regulation of sphingolipid-signaling by neutral ceramidase

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Neutral ceramidase is a type II integral membrane protein which is occasionally secreted into the extracellular milieu after the processing of its N-terminal anchor. We found that when overexpressed in CHOP cells, neutral ceramidase hydrolyzed cell-surface ceramide which increased in amount after the treatment of cells with bacterial sphingomyelinase or doxorubicin, leading to an increase in the cellular level of sphingosine and sphingosine 1-phosphate (S1P). On the other hand, knockdown of the endogenous enzyme by siRNA decreased the cellular level of both sphingolipid metabolites. The enzyme also seems to participate in the hydrolysis of serum-derived ceramide in the vascular system. Significant amounts of sphingosine as well as S1P were generated in the cell-free conditioned medium of ceramidase transfectants, compared to mock transfectants. A S1P receptor, S1P₁, was internalized much faster by the treatment of S1P₁-overexpressing cells with conditioned medium of ceramidase transfectants than that of mock transfectants. Interestingly, knockdown of the enzyme by anti-sense morpholino oligonucleotide inhibited the S1P-mediated angiogenesis during zebrafish embryogenesis. These results clearly indicate that the enzyme is involved in ceramide metabolism at the plasma membrane and in extra milieu, which regulates the S1P level and consequently S1P-mediated signaling.