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Regulation of DHS1P release in F9 mutant cells by fumonisin-B1

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Objectives

Sphingosine 1-phosphate (S1P), a bioactive ligand is produced by sphingosine kinases (Sphk) by combining sphingosine with ATP in cells. It binds to its receptors, S1PR1-S1PR5 and exerts several pathophysiological functions in vascular and brain systems. There has been a common question on how S1P is released outside through cell membrane. The main goal of our study was to determine the regulation mechanism of dihydrosphingosine 1-phosphate (DHS1P) release from F9 teratocarcinoma mutant cells.

Materials and Methods

We used a specific mutant (F9-12) of F9 cells over-expressing Sphk1 and showing null of S1P lyase. The concentrations of S1P, DHS1P, sphingosine, dihydrosphingosine (DHS) and Sphk activity were measured by HPLC system after orthophthalaldehyde (OPA) derivatization. Flow cytometry was used to count apoptotic cells number.

Results and Discussion

Fumonisin B1 (FB1), ISP-1, dimethylsphingosine (DMS) and inhibitors of ABC transporters were treated to F9-12 cells to see the changes in DHS1P release. Fumonisins are produced by fusarium verticillioides and other fusarium that are contaminated on corn. The major target of FB1 in mammalian cells is ceramide synthase in sphingolipid de novo synthesis. FB1 treatment (50 uM) for 8 hrs exhibited concurrent increases of DHS (from 123.0± 29.2 pmol/mg protein to 9570.7 ±935.7 pmol/mg protein: 77.8 fold increase) and DHS1P (from 142.0 ± 17.8 pmol/mg protein to 5008.5 ±430.8 pmol/mg protein: 35.3-fold increase) in F9-12 cells. In this conditions, the increased DHS and DHS1P also efficiently released outside (DHS, 3.6-fold increase; from 1.0 ± 0.4 pmol/10^6cells to 3.6±0.6 pmol/10^6cells, DHS1P, 12.4-fold increase; from 2.8 ± 1.6 pmol/10^6cells to 34.8 ±19.0 pmol/10^6 cells, respectively). Therefore, we knew FB1 treatment increased sphingolipids release and accumulation in cells. ISP-1 (20 nM, a specific inhibitor

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of serine palmitoyltransferase) and DMS (10 uM inhibitor of Sphks) treatment largely attenuated the accumulation of DHS and DHS1P in both inside and outside of cells. FB1 treatment induced a small percentage of apoptotic cells, indicating the releases of DHS and DHS1P were mediated by passing through cellular membrane. We further investigated the effects of ABC transporters on DHS1P release. By using ABC transporters inhibitors, verapamil, MK-571 and glyburide, the involvement of ABC transporters on DHS1P release was verified. Conclusively, DHS1P release may be regulated by the rate of sphingolipid biosynthesis and Sphk activity. We now tried to clarify which classes of ABC transporters were involved in sphingolipid release in cells.